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AEROBIC MESOPHILIC SPOREFORMING BACTERIA

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[1] On military leave.

[2] The preliminary work was done by F. E. Clark and N. R. Smith. Owing to a change in the former's assignment, the work was continued and completed with the help of R. E. Gordon. The writers wish to express their deep appreciation to Charles Thom, formerly principal mycologist, in charge of the then Division of Soil Microbiology, for his encouragement and helpful suggestions during the course of the work; to R. S. Breed, of the New York State Agricultural Experiment Station; and to others who have shown so much interest in the subject. The writers also wish to acknowledge the great assistance given by J. Roger Porter, University of Iowa, who turned over to them a collection of sporeforming bacteria that he had collected from American and European laboratories. They also wish to thank the American Type Culture Collection, the National Collection of Type Cultures (London), and the many individuals who donated named cultures.

SUMMARY

Aerobic mesophilic sporeforming bacteria are usually considered as inhabitants of the soil. Because of the formation of heat-resisting spores, they may be found in practically any location. They may cause the spoilage of food and other materials and may be found even in medical work, where they are sometimes suspected of being associated with certain pathological conditions. During the past few years much interest in this genus has arisen because of the discovery of antibiotic substances produced by certain species.

Ford and his coworkers attempted to classify this genus in 1916, but lack of knowledge of the different stages of growth and the variation in bacterial cultures, as well as lack of adequate differential tests, left their work incomplete. The result was that bacteriologists—medical and agricultural—often attached a new name to a culture that they had isolated, believing it to be a new species. This, combined with the distribution of mislabeled cultures, led to such confusion that no one could identify more than a very few of the species.

A total of 621 cultures of aerobic mesophilic sporeforming bacteria were studied and placed in 3 groups on the basis of morphology and physiology. Of this number, 363 bore specific names; the remaining 258 were either received unnamed or were isolated by the writers.

Of the three groups the first is the largest, comprising about 77 percent of the cultures studied. It includes *Bacillus megatherium*, *B. cereus* and varieties, *B. subtilis* and varieties, *B. pumilus* (formerly called *B. mesentericus* in America), and the lesser known species *B. coagulans*, *B. firmus*, and *B. lentus*. Studies on dissociation were made on *B. mycoides* and its biotype *B. praussnitzii*, which resulted in placing *B. mycoides* as a variety of *B. cereus*. Comparative studies indicated that *B. anthracis* differed from *B. cereus* in being pathogenic and nonmotile. Since both are mutable characters, *B. anthracis* has been placed as a variety of *B. cereus*. Dissociation of the black *B. niger* and the black *B. aterrimus* to colorless forms indistinguishable from *B. subtilis* proved that they were only varieties of the latter.

Group 2 contains about 19 percent of the cultures. It includes *B. polymyxa*, *B. macerans*, *B. alvei*, *B. circulans*, *B. brevis*, and *B. laterosporus*. Group 3, the round-spored group, with only about 4 percent of the cultures, contains *B. sphaericus* and varieties and *B. pasteurii*. *B. fusiformis* has been placed as a variety of *B. sphaericus* because of its production of urease. *B. rotans* and *B. loehnisii* are also tentatively placed there as varieties. The apparent unimportance of groups 2 and 3 because of the small number of cultures studied may bear no relation to the occurrence of the species of these groups in nature.

The Voges-Proskauer reaction proved to be an important and a reliable test for separating some species if certain modifications of the usual method were followed. The storage of fat and the fermentation of carbon compounds were studied extensively and were found valuable, provided that liberal interpretations were made of the observations. Of special interest was the ability of certain strains to produce adaptive enzymes so that carbohydrates not regularly attacked could be utilized. In many cases these cultures lost this ability

after a few months' storage on nutrient agar in the absence of the carbohydrate.

Agglutination by antiserums produced with whole cells as antigens was too specific for taxonomic use. Bacteriophage, although too specific in some instances, was very useful in others, especially in the study of *B. megatherium*, *B. cereus* and varieties, *B. pumilus*, and *B. brevis*.

It was found that many species previously described and separated from others because of a rough, folded, mucoid, rhizoid, or pigmented growth were merely growth phases or varieties of established species. It was also found that still other species characterized by some special physiological trait were only biotypes of the "parent species." Consequently, a large number of adequately described named cultures have been placed in the synonymy of the various species. If the original description was not adequate to characterize the organism, it was assigned to a species on the basis of the culture as received. This resulted in a reduction from about 120 to 15 specific names.

A dichotomous key for use in identifying cultures of the mesophilic aerobic sporeforming bacteria has been prepared in which two characters, so far as possible, are used at each separation instead of one. A variation or misinterpretation of one of these should not, therefore, lead to a wrong conclusion.

NEED FOR CLASSIFICATION

During the 27 years since Ford and his associates (*60*, *61*) [3] published their work on the sporeforming bacteria, very little progress has been made in the classification of this group. In the meantime new species have been described briefly without comparing them with those already existing in the literature. Often there was only a simple isolation to which was ascribed some special character, as the ability to grow upon a certain medium or to produce a certain compound, and no attempt was made to observe closely related strains for that same property. The futility of attempting to arrange species so badly described is illustrated by the classification of the genus *Bacillus* in the fifth edition of Bergey's Manual (*6*).

For a number of years it had been obvious that this genus was poorly arranged and needed thorough study. The stimulus to attempt this came when a culture of *B. siamensis* Sirabaed (*94*) was studied and was identified as a strain of *B. cereus* (*17*), the most common of the sporeformers occurring in soil. Since the best method of approach seemed to be by a study of authentic cultures, requests for transplants of various members of the genus were sent to individuals, laboratories, and institutions, resulting in a collection of 363 named strains. In addition, 258 unnamed cultures and isolations also were studied.

As the number of strains in the collection increased and information on them accumulated, it became evident that certain characters were comparatively stable, whereas others were so variable as to be taxonomically useless. The limits of variability in certain characters have been established for some species. This has resulted in the discard of some old specific names of the genus *Bacillus* and in the

[3] Italic numbers in parentheses refer to Literature Cited, p. 102.

demotion of other species to the rank of varieties. In many cases it has not been possible to place definitely the named organism, because of inadequate description or lack of agreement between the culture obtained and its characters as originally given.

It is common knowledge that cultures may pass through various growth stages (rough, smooth, mucoid, dwarf, and other states) and may vary in pigment formation, carbohydrate fermentation, and, to a lesser extent, even as to pathogenicity. In fact, it is believed by an increasing number of bacteriologists that any character may vary to some extent. It becomes necessary, therefore, to rely upon a group of properties for specific differentiation, or what might be called a species pattern. The pattern of one species may overlap a closely related species, giving rise to intermediates. One should not, however, be able to change a culture from one pattern to another; in other words, one species should not change into another, either spontaneously or by changes induced in the laboratory. The classification proposed in this paper is based upon such a species concept. In some cases justification for the arrangement has been obtained by studies on dissociation; in others, through the occurrence in the collection of cultures representing certain stages or varieties that indicate their relations.

The choice of a name to represent a species that is now shown to have other named species as varieties or dissociants presents a problem. One solution would be to select for the species the name that appeared first in the literature (priority) without regard to systematic relations, the stability of the characters of the species, or the suitability of the name. The authors have reason to believe that this view will be taken by some. To them priority means everything, and they argue that without adhering strictly to the rules of priority only confusion and more confusion will result. The authors, however, share the experience of other workers in finding that bacterial strains will lose a property much more readily than gain one. It has not been unusual to find that a strain previously named because of its fermentation of lactose, its slimy or folded growth, its pigmentation, its pathogenicity, or other characteristics no longer possesses the distinctive trait after long cultivation under laboratory conditions. In order to place it correctly, therefore, the taxonomist would have to depend on the culture's history rather than on the results of his study of this and other related strains.

Another solution, presented here, puts the natural relations of the dissociants and variants ahead of priority; not, however, disregarding the latter entirely. It is based upon the knowledge that any species may exhibit different stages of growth (rough, smooth, mucoid) and that it may vary in its physiology and morphology within certain limits. An example will illustrate what is meant.

It will be shown below that *B. cereus* possesses quite a wide range of characters and that some of its variants and biotypes have been given names as species. For instance, certain strains ferment lactose and have been called *B. albolactis*, *B. lactis*, and *B. lacticola;* others produce a yellowish-green fluorescent pigment and have been called *B. cereus-fluorescens* or *B. fluorescens* (not *Pseudomonas fluorescens*); others produce a rhizoid growth on agar, and if they do not ferment lactose they are *B. mycoides*, if they do ferment it they are *B.*

praussnitzii. Each one of these characters may be easily lost during studies on dissociation, and the resulting cultures cannot be distinguished from the typical *B. cereus*. The writers consider, therefore, that *B. cereus* is a "parent" or "basic" species. In such cases they propose that a culture bear as its specific name the earliest valid name given to its "parent" species.

With this concept of a basic species in mind, the question arises, When is a variant a variety and when is it a biotype? The writers do not know of any rules governing such nomenclature, and for the time being they prefer to leave the designation of these an open question. In general, they have considered a variety as possessing a character that may be lost but not gained in culture, whereas a biotype may lose or gain it. For instance, *B. mycoides* is called a rhizoid variety of *B. cereus*, because it loses that kind of growth when grown in a large volume of broth and has not been known to revert to type. On the other hand, *B. albolactis* and similarly named cultures lose the ability to ferment lactose after long cultivation in its absence but may be induced to do so again by appropriate means. Strains of *B. cereus* isolated from soil very rarely ferment lactose. A number were induced to do so, but most of them lost that property in a few months. All these might be called lactose-positive variants or biotypes. The latter is used in this paper.

It may not be amiss to state here that before an investigator names a new species he should not only isolate and study a number of isolations but he should also determine the limits of variability of his cultures. In disregarding such a procedure, it may happen that a species, named because of some supposedly special character and no longer possessing it, will be discarded as a contamination rather than be kept as a reversion to the parent species. This is one of the factors that has caused so much confusion in the genus under investigation. In addition, inadequate descriptions, as well as the distribution of mislabeled or contaminated cultures, have played their part in making it almost impossible for the average bacteriologist to identify correctly a mesophilic aerobic sporeformer.

The taxonomist is naturally limited by his collection. If he does not have enough cultures of a species for study, his knowledge of that species will be incomplete. The species of two subgroups, *B. megatherium–B. cereus* and *B. subtilis–B. pumilus* given below, are fairly well represented in this collection. There are enough intermediate strains to show the relations of the different species to each other. There are natural varieties or biotypes of each species, and several of these have been dissociated further to justify their incorporation in the species. Strains of the two remaining subgroups, *B. circulans–B. brevis* and *B. sphaericus–B. pasteurii*, are found in comparatively small numbers in nature. Since they grow more slowly and less conspicuously, many of the named strains of these two subgroups were found to have been contaminated and overgrown by *B. subtilis* or some other organism. For these reasons this collection does not contain enough strains to give as clear a taxonomic picture of these two groups as is desirable. Nevertheless, the cultures are described and the possible species and subspecies are pointed out with the acknowledgment that they might have to be revised after the study of a larger collection. Although much has been done, this re-

port is not intended to be the final word on this genus, the members of which are so often found in all fields of bacteriology.

STUDIES ON DISSOCIATION

Although studies on dissociation are placed first in this publication, they really followed the intensive observations made on the particular species. It was not possible to subject each species to dissociative studies, so it may be that some organisms now listed as species will be found, on more intense study, to be varieties. It is also possible that some investigators would prefer to call certain strains varieties instead of biotypes, as is done in this publication. The authors do not consider it very important whether they are considered biotypes or varieties, so long as the relation to the parent species is recognized. They are very much opposed, however, to designating a species on some character that may disappear spontaneously or be lost by dissociation.

Dissociation of Bacillus mycoides

HISTORICAL BACKGROUND

Bacillus mycoides Flügge is widely known and easily recognized because of its unique colony formation. In 1881 Koch (*55*) was undoubtedly referring to *B. mycoides* when he mentioned an unnamed bacillus, very resistant to heat, whose colonies were like interwoven mattings of roots. Five years later, Flügge (*35*, *p. 324*) named and described *B. mycoides* as a sporulating rod resembling the anthrax bacillus in size, with oval, central spores and colonies of tangled, rootlike, ramifying threads.

Variation in the colonial form of *B. mycoides* was first reported by Nadson and Adamovic in 1910 (*79*), although their observation that the unusual colony resembled an actinomycete was not substantiated by later workers. In 1927 Nyberg (*82*) found that five of six cultures of *B. mycoides* when transferred in broth or peptone water for several months showed some typical rhizoid colonies and also some of a very different appearance. He stated that these atypical colonies closely resembled those of *B. mesentericus*. In a later paper (*83*) he continued his earlier work with a larger number of cultures of *B. mycoides* and their variants and was not able to separate them physiologically from cultures of *B. mesentericus* and *B. subtilis*. In this connection, it should be remembered that *B. cereus* was often called *B. subtilis* at that time (*21*). Perhaps Nyberg's strains of the latter were really the former. Oesterle and Stahl (*84*), Stapp and Zycha (*102*), Brunstetter and Magoon (*9*), and Lewis (*67*, *69*) observed a change of colony form by *B. mycoides* after growth in liquid medium.

In 1931 Dooren de Jong (*29*) described a sporeforming bacillus and named it *B. undulatus*. The two cultures bearing this name, which the writers received from Dooren de Jong through Porter, are *B. cereus*, and the original description is clearly that of the same species. In 1933 Dooren de Jong (*30*) also observed the nonrhizoid colonies of *B. mycoides* and stated that these smooth atypical forms were identical with *B. undulatus*. Soriano and Soriano (*100*) recog-

nized what Dooren de Jong and others had missed; namely, that the atypical strains of *B. mycoides* were in fact *B. cereus*, described by Frankland and Frankland in 1887 (*38*). Taxonomic studies of these two species reported here lead to the same conclusion.

DISSOCIANTS OBTAINED

In the writers' collection there are 19 cultures named *Bacillus mycoides* and one named *B. praussnitzii*, the latter said to differ from *B. mycoides* only in its ability to ferment lactose (*60, p. 495*). These strains have been cultivated on nutrient agar in this laboratory from 3 to 20 years and during that time have given no indication of departure from their characteristic colony form. After preliminary observations had shown that variation might occur in a fairly large volume of broth, a colony of each of the 20 rhizoid cultures was picked from nutrient agar plates into flasks containing 100 cc. of nutrient broth. These were held at 28° C., and plates of nutrient agar made between the third and tenth day. The plates showed varying numbers of dense, softer, nonadherent colonies mixed with the typical mycoid ones (pl. 1, *A*).

The variant colonies have the characteristic appearance of colonies of *B. cereus* described by different writers as resembling ground glass, galvanized iron, or watered silk, due to the adherence of the cells in long, parallel chains. The chains do not cling and twist together into threads as in the rhizoid colony but are spread out in a looser, wavy arrangement, as though they had been combed. It is impossible to distinguish between the variant colonies of *B. mycoides* and the colonies of *B. cereus*.

Daily serial transfers of *B. mycoides* in tubes containing 5 cc. of broth caused colony variation in 8 to 10 days, whereas cultures grown in single tubes of broth did not dissociate until 4 to 6 weeks old. Cultures inoculated into beef broth diluted to only 10 percent of its normal nutrients also dissociated but not so quickly as in a richer broth. On the other hand, cultures transferred serially 50 times on nutrient agar did not lose their character of rhizoid growth, although 2 out of 5 cultures dissociated on glucose-beef agar after 30 serial transfers. The rhizoid form will, therefore, not always remain stable when cultivated on solid medium, but dissociation is difficult and infrequent compared with the ease of the change in a large volume broth culture.

VITAMIN REQUIREMENTS

The work of Robbins (*88*) on the growth requirements of a strain of *Fusarium avenaceum* suggested that a lack of nutritional elements in broth as compared with agar might be a factor in the dissociation of *Bacillus mycoides*. Like the *Fusarium* culture, this organism fails to grow in a solution of mineral salts and glucose, but will grow on the same solution solidified or semisolidified with agar. Since it was the biotin in the agar that was effective with Robbins' culture, the following components of the vitamin B complex were used: Riboflavin, thiamin hydrochloride, nicotinic acid, and calcium pantothenate. Each vitamin was tested separately by adding 0.1μg., 1μg.,

5μg., and 10μg. to a tube of glucose mineral solution. *B. mycoides* failed to grow with any one of the vitamins in the concentration used. On the other hand, 1μg. of biotin added to a tube of the medium was enough for growth and acid formation from the glucose. This would indicate that the growth-promoting substance in agar is biotin or something contained in the biotin.

It is assumed that some biotin is present in nutrient broth, because good growth occurs even when the broth is diluted. Nevertheless, it was thought advisable to test the effect of the addition of biotin to diluted broth. One microgram of biotin in sterile solution was added to each tube of nutrient broth, which had been diluted to 10 percent and 25 percent of its original strength. The cultures of *B. mycoides* inoculated into this biotin medium were examined at 5 and 7 days. Variant colonies were found on the plates of most cultures at 5 days and on the plates of all cultures at 7 days. These results are comparable with those obtained above without biotin. It has, therefore, no effect on dissociation.

EXAMINATION OF DISSOCIANTS

Microscopically the cells of both *Bacillus mycoides* and *B. cereus* are large rods, 0.9μ to 1.2μ wide, with squared ends and oval centrally placed spores that swell the sporangium only slightly if at all. They normally contain a large number of vacuoles, which give the lightly stained cells a foamy appearance. Generally the rods of *B. mycoides* are longer and a trifle thinner than those of *B. cereus*, form longer chains, and do not sporulate so profusely. Although many of the cultures of *B. cereus* in this collection form spores quickly and abundantly, the extent of sporulation varies with the strain. The cells of the dissociants of *B. mycoides* when first isolated generally resembled those of the rhizoid stage in size and formed no spores. With continued transfer on agar they became shorter and thicker, appeared singly or in short chains, and slowly gained the ability to form spores. Three years after the separation of the non-mycoid cultures of *B. mycoides* a comparison of the numbers of spores seen in cultures of these dissociants and of *B. cereus* was made and is presented in table 1. The cultures show that *B. cereus* and the

TABLE 1.—*Comparison of numbers of spores formed by cultures of Bacillus cereus and by the nonrhizoid dissociants of B. mycoides*

B. cereus	Spores in—		Dissociants of *B. mycoides*	Spores in—	
	24 hours	48 hours		24 hours	48 hours
No. 410	+++[1]	++++	No. 233(C) [2]	+	+
No. 538	+	+	No. 273(C)	++++	++++
No. 539	+	++	No. 273(16)	+	++++
No. 540	+	+	No. 306(C)	++++	++++
No. 542	++	++++	No. 306(18)	+	+
No. 543	++	++	No. 317(C)	++	++++
No. 544	+	+++	No. 317(4)	+	++
No. 545	++	+++	No. 317(16)	++++	++++
No. 589	+	++	No. 318(C)	+	+
No. 599	++++	++++	No. 319(C)	+++	+++

[1] + indicates an occasional spore; ++, few spores; +++, numerous spores; ++++, spores in the majority of the rods.

[2] The designations in parentheses identify the various dissociants.

A, Dissociation of *Bacillus mycoides:* Plate from a 10-day-old broth culture, showing typical colony above and variant colonies below. *B*, Range of pigmentation on potato found in dissociating cultures of *B. aterrimus: a*, Typical black pigment; *b*, gray; *c*, pink; and *d*, cream-colored.

variants of *B. mycoides* cannot be separated on the basis of sporulation. No. 317 in table 1 also illustrates that dissociants of the same rhizoid culture of *B. mycoides* do not form like numbers of spores. A large number of physiological tests on *B. mycoides*, on its dissociants, and on *B. cereus* failed to show any consistent difference that might be used for separating them.

LYSIS OF DISSOCIANTS BY BACTERIOPHAGE

A pure-line bacteriophage was isolated from soil and developed against *Bacillus cereus* No. 201. (See section on Bacteriophagy, p. 28.) It lysed all strains of *B. cereus* in the collection but was inactive against the rhizoid form of *B. mycoides* and *B. praussnitzii*. After dissociation, however, the variants of these two species were readily dissolved by this phage. Another bacteriophage was developed against *B. mycoides* No. 319 that did lyse the rhizoid forms; it also dissolved the dissociants and cultures of *B. cereus*. These observations provide more information that the relationship of *B. mycoides* and *B. cereus* is very close.

STABILITY OF THE DISSOCIANTS

The nonrhizoid stage of *Bacillus mycoides* is very stable, as only one of the 33 variant cultures selected for study returned spontaneously to the rhizoid form. This culture reverted very soon after its separation, and it may not have been fully dissociated. The remaining 32 cultures were carried on agar along with the stock cultures for 3 years, plated from time to time, and carefully examined for rhizoid colonies, but none were ever found.

In order to stimulate reversion, cultures of the dissociants of *B. mycoides* and also some cultures of *B. cereus* were grown on different mediums under varying conditions of pH, oxygen tension, temperature, humidity, and surface tension and in immune serums and bacteriophage. No mycoid colonies were ever found, although the cultures often became very rough and arborescent. Reversion should be possible, but the conditions necessary for this have not been found.

Dissociation of Bacillus praussnitzii

Bacillus praussnitzii has been said to resemble *B. mycoides* except for its active fermentation of lactose (*60, p. 495*). The writers can substantiate this. They found also that it dissociated in the same way as *B. mycoides* and that its variants fermented lactose. These could not be differentiated from the so-called *B. albolactis* that in all other respects is identical with *B. cereus*. Since the utilization of lactose is an uncertain property (see section on fermentation studies below), it is recommended that these lactose-positive strains be considered as biotypes rather than as varieties. *B. praussnitzii* and *B. albolactis* become, therefore, biotypes of *B. cereus* var. *mycoides* and *B. cereus*, respectively.

Dissociation of Bacillus aterrimus

The similarity between *Bacillus subtilis* (*B. vulgatus*), *B. aterrimus*, and *B. niger* was noted early in this work. Clark and Smith

(*20*) reported that *B. aterrimus* formed a black pigment only upon mediums containing a carbohydrate, whereas *B. niger* blackened only mediums containing tyrosine. Attempts to develop dissociants that would not be black by aging in acid, neutral, and alkaline nutrient broth were not successful. Later, in discussing these results T. Gibson [4] told the writers that certain of his freshly isolated cultures of *B. aterrimus* had lost their ability to form pigment after growing in glucose broth. To confirm Gibson's statement *B. aterrimus* No. 740 was transferred serially in glucose broth at 28° C. Occasionally a culture was obtained that was not black when transferred to potato, but if it was transferred again to potato it would revert to the black form. Potato plugs were used, because they are especially favorable for the development of the black color.

A more selective procedure, therefore, was tried (table 2). The blackened potato culture was plated on glucose-nitrate agar, and

TABLE 2.—*Loss of the black pigmentation of Bacillus aterrimus by plating and selection, 1941*

Time of testing	Color of cultures on potato [1]			
	Black	Gray	Pink	Cream
	Number	*Number*	*Number*	*Number*
May 1	* 1			
15	4	0	* 1	0
26	5	0	* 2	0
June 9	5	0	* 5	0
23	2	4	4	* 3
July 5	0	3	6	* 3
15	0	3	5	* 2
Aug. 6	0	3	2	* 6
18	0	0	0	*10
28	0	0	0	12

[1] One of the starred (*) potato cultures was selected each time for plating on glucose-nitrate agar. Several of the least pigmented colonies were picked to glucose-nitrate agar slants, and those that blackened the agar least were transferred to potato.

several colonies that blackened the agar least or not at all were picked to slopes of glucose-nitrate agar. Tubes showing no pigment, or the least quantity of it, were transferred to potato plugs. Of the potato cultures of May 15, four were black and one pink. This pink culture was plated on glucose-nitrate agar; colonies were again selected and subcultured to agar slopes and next on potato. This time five black and two pink cultures resulted. One of the two pink cultures was plated and the selective process was repeated. In this way the black cultures were gradually eliminated. The cream-colored cultures of August 18 and 28 (1941) did not revert to the black form during a year's observation. Plate 1, *B*, is from a photograph of four potato plugs inoculated with subcultures of a dissociating culture of *B. aterrimus* No. 740. These four daughter cultures represent all the pigments found, black to cream, with the intermediate gray and pink.

The ease with which dissociation of *B. aterrimus* can be accomplished as a variable character is illustrated in table 3. In this experiment the procedure differed slightly from table 2. Six strains

[4] By oral communication, September 1939.

were transferred serially 50 times on slopes of glucose-nitrate agar and then plated on the same medium. Colonies were selected and transferred to potato plugs. The potato culture that showed no black pigment or the least degree of it was plated again. After incubation, colonies were picked to potato and the process repeated. Culture No. 624 lost its black pigment entirely during the serial transfer on glucose-nitrate agar, whereas the dark pigment of Nos. 259 and 261 was eliminated only after 2 and 3 platings, respectively. The other 3 cultures showed varying degrees of dissociation, Nos. 230 and 276 reverting to some extent to the black form.

TABLE 3.—*Variation of pigmentation of cultures of Bacillus aterrimus after 50 serial transfers on glucose-nitrate agar, followed by plating, colony selection, and growth on potato, 1942*

Cultures of *B. aterrimus*	Pigment on potato [1] after—											
	First plating, Jan. 17				Second plating, Feb. 2				Third plating, Feb. 12			
	Black	Gray	Pink	Cream	Black	Gray	Pink	Cream	Black	Gray	Pink	Cream
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
No. 230	0	0	0	*8	0	2	0	*4	0	2	0	3
No. 259	1	0	*3	0	0	0	*6	0	0	0	6	0
No. 261	0	1	*3	0	0	3	*3	0	0	0	2	3
No. 275	2	2	0	*2	0	2	0	*4	0	3	0	2
No. 276	0	*4	0	0	*6	0	0	0	2	1	1	0
No. 624	0	0	*8	0	0	0	*6	0	0	0	5	0

[1] One of the starred (*) potato cultures made after the first plating was selected for plating on glucose-nitrate agar (second plating). Several colonies were picked to potato, and one of those starred was plated again.

Dissociation of Bacillus niger

For dissociating *Bacillus niger* other procedures were followed, mainly to show that a bacteriologist is not limited to a single technique. Slants of nutrient agar containing 0.05 percent of tyrosine were inoculated and incubated for 2 weeks at 43° C. At the end of that time, duplicate tubes were inoculated, one placed at 28° and the other held at 43°. After 2 weeks, the process was repeated, always using the tube from the higher temperature as the inoculum.

Three of the twelve strains tested (Nos. 223, 229, and 650) lost their ability to form the pigment after 12 to 18 transfers. The 6 other strains from which stabilized colorless dissociants were not obtained during the same time definitely showed the inhibitive effect of the higher temperature. After 1 or 2 transfers no blackening occurred at 43° C. Tubes inoculated from the higher temperature culture and held at 28° showed a great reduction in the degree of the pigment, sometimes lacking it entirely. On a second transfer to 28°, however, they would revert to the pigmented form.

Another procedure was also tried. Glucose-nutrient broth cultures were transferred serially at 43° C. from two to eight times, plated on tyrosine-nutrient agar, colonies picked, and the tubes held at 28°. Three cultures (Nos. 356, 651, and 655) responded to this treatment, whereas five did not lose the pigment during this short test. Owing to interruption of the work, the three colorless dissociants were not

fully tested for reversion; it is believed, however, that they were stable.

It was noted in the development of the above dissociants that the intermediate cultures, with one exception, merely lost the pigment gradually without going through gradations of pink as noted with *B. aterrimus.* In the case of No. 356, an orange-colored intermediate appeared. It was not stable, giving rise to the usual cream-colored growth typical of the dissociants and of *B. subtilis.* Whether pink, red, or orange intermediates have any bearing upon the nature of the pigment cannot be stated at this time. Muschel (*78*) thought that the pigment of *B. aterrimus* resulted from a polyphenyloxydase blackening of carbohydrate-condensation products. It might differ, therefore, from the melaninlike pigment of *B. niger*, which is probably formed through the tyrosinase system.

Previous to these dissociation studies, cultures of *B. aterrimus*, *B. niger*, and *B. subtilis* were thoroughly studied, using about 60 different tests. No property was found, except the pigmentation, that could be used to separate them. Without their ability to blacken mediums, the 52 colorless dissociants developed from 19 strains of *B. aterrimus* and *B. niger* could not be differentiated from *B. subtilis.* It is recommended, therefore, that *B. aterrimus* and *B. niger* be reduced from species rank to that of variety and that they be known as *B. subtilis* var. *aterrimus* and *B. subtilis* var. *niger*, respectively.

FACTORS AFFECTING THE PRODUCTION OF ACETYLMETHYLCARBINOL

Use of the Voges-Proskauer Test

The Voges-Proskauer reaction (*113*), demonstrating the presence of acetylmethylcarbinol (acetoin), has long been used to differentiate *Escherichia coli* from *Aerobacter aerogenes*, but its use in the study of the aerobic sporeformers seems to have been limited. Desmots (*28*) noted that *Bacillus mesentericus*, *B. subtilis*, and *Tyrothrix tenuis* produced acetylmethylcarbinol, which was corroborated later by Harden and Norris (*47*). Lemoigne (*64*) studied the decomposition of sugar by *B. subtilis* in more detail. He proved that acetylmethylcarbinol was formed from the sugar in the medium, amounting in some cases to more than 0.3 gm. per 100 ml. of medium containing 4 percent sugar. He also noted that the quantity of acetylmethylcarbinol reached a maximum and then decreased, even to its complete disappearance. He considered it a special type of fermentation that was of great importance in nature.

Early in this work it was noticed that members of the *B. subtilis–B. pumilus* group produced acetylmethylcarbinol by the methods recommended (*1*, *99*). The V-P (Voges-Proskauer) test was, therefore, used in the preliminary classification (*95*) as a means of separating these species from others. Subsequent tests on *B. cereus* and its variety *mycoides* (see section on dissociation of *B. mycoides*) and on *B. alvei* showed that some, but not all, strains of these bacilli would also produce acetylmethylcarbinol. It was also noticed that the V-P reaction varied with different batches of broth. These observations led to a study of the factors influencing the production

of acetoin with a view of standardizing a test that might serve for all species in this genus. In the past, the greater emphasis was placed on the detection of acetoin rather than on its production and, furthermore, practically all the work has been with the *Aerobacter aerogenes* group. The method described here makes this one of the easiest and most reliable tests for separating species of the genus *Bacillus.*

Steaming the Medium

Since uniform results were not obtained with different batches of broth, the first factor studied was the effect of autoclaving. Davis and Rogers (*26*) found that fructose, dextrose, arabinose, and lactose were changed more in a buffer solution than in distilled water and consequently recommended "momentary heating," i.e., raising the temperature to 120° C. for 10 minutes and then cooling slowly. With this in mind the following test was made. Standard V-P broth [5] was prepared and divided into four parts. One part was tubed and autoclaved at 15 pounds' pressure for 40 minutes (long sterilization), another for 20 minutes (regular sterilization), and one for 12 minutes (short sterilization). The fourth part was sterilized by filtering through a Pasteur-Chamberland bougie, porosity L2, using the technique of Desai (*27*). Five ml. of the filtrate was pipetted into sterile 18-mm. test tubes and incubated to test for sterility.

Table 4.—*Comparison of the effect of sterilization of medium by steam and by filtration on production of acetylmethylcarbinol* [1] *in standard Voges-Proskauer broth; 3 days at 37° C.*

Culture	Autoclaved 15 pounds for—			Filtered
	40 minutes	20 minutes	12 minutes	
B. cereus:				
No. 201	−	−	±	+++
No. 202	?	?	±	+++
No. 232	−	?	?	++
No. 305	−	±	+	+++
B. subtilis var. *aterrimus* No. 659	+	+	++	++
B. subtilis var. *niger* No. 228	++	++	++++	++++

[1] ++++ indicates very strong; +++, strong; ++, weak; +, positive, but very weak; ±, doubtful; ?, very doubtful; —, no reaction.

Table 4 shows that heating the medium rendered it quite unfit for the formation of acetylmethylcarbinol by *Bacillus cereus.* On the other hand, *B. subtilis* vars. *niger* and *aterrimus* were less affected. In addition, it was found that autoclaving the filtered medium gave the same results, showing definitely that heating the medium caused changes that interfered with the production of acetoin.

Many other similar tests showed that *B. alvei* and *B. cereus* var. *mycoides*, like *B. cereus*, found the heated medium unsuitable for the formation of acetoin. Intermittent sterilization (flowing steam) also was used, but this was no better than the regular autoclaving.

[5] Composition: Proteose-peptone 7 gm., dipotassium phosphate 5 gm., glucose 5 gm., and distilled water 1,000 ml.

In spite of the good showing of the filtered medium, it was decided that, since this technique might be objectionable and laborious to some workers, other factors should be investigated before recommending its adoption.

In the next experiment, the three components of the medium were made up in triple-strength solutions. Different lots of mediums were then prepared by taking aliquots and—

(1) Combining the three and autoclaving; regular medium.
(2) Autoclaving each medium separately and combining aseptically.
(3) Combining the buffer and glucose and autoclaving; then adding the autoclaved peptone.
(4) Combining the peptone and glucose and autoclaving; then adding the autoclaved buffer.
(5) Combining the peptone and buffer and autoclaving; then adding the autoclaved glucose.
(6) Combining the peptone, buffer, and glucose and sterilizing by filtration.

The results can be briefly summarized. Mediums Nos. 1 and 3 were discolored and proved to be unsuitable for the production of acetoin. On the other hand, Nos. 2, 4, and 5, in which the glucose and buffer were not heated together, were satisfactory and comparable with No. 6, which was sterilized by filtration. This experiment showed, therefore, that heating the medium containing K_2HPO_4 and glucose changed the medium and thus interfered with the formation of acetoin. This fact was more clearly brought out by autoclaving the medium already sterile by filtration and inoculating with *B. alvei* No. 395 and *B. cereus* No. 232. The former failed to show any acetoin in the heated medium, although it produced a 3+ reaction in the filtered control. *B. cereus* formed only a trace in the autoclaved filtered medium and a 3+ in the control.

The question then arose whether the buffer was really necessary. The previous mediums Nos. 1, 4, and 6 were compared with another that contained no buffer (K_2HPO_4). The results showed that *B. subtilis* and *B. cereus* yielded much more acetoin without the buffer, but that *B. alvei*, on the other hand, did not form it so regularly nor in so great a quantity as it did on the filtered medium or when the buffer was added separately.

At first it appeared as if two mediums would have to be used, one with K_2HPO_4 and one without. In an attempt to obviate this, another experiment was set up to determine whether some other buffer or sodium chloride would fulfill the requirements. For this test, double-strength proteose-peptone glucose solution was sterilized and combined aseptically with an equal quantity of autoclaved 1-percent solution of either Na_2HPO_4, $(NH_4)_2HPO_4$, or NaCl. In addition, two other batches of peptone glucose broth, one with and one without 0.5 percent NaCl, were made and autoclaved in the usual manner. Some typical results given in table 5 show that Na_2HPO_4 gives the same result as K_2HPO_4, as might be expected, whereas $(NH_4)_2HPO_4$ was very poor, even in the case of *B. alvei*. Sodium chloride, however, proved to be as good as potassium or sodium phosphate for this species; it was not harmful to the others and could be incorporated into the medium before sterilization, thus simplifying the procedure. A large number of cultures were used in comparing this medium with the standard medium and with one without salt. In all cases the modified medium was as good or better, depending upon the

organism tested. Similar results were obtained with four strains of *Aerobacter aerogenes* in the writers' collection. It is therefore recommended that NaCl be substituted in equal quantity for the K_2HPO_4 in the standard medium used for the V-P test.

TABLE 5.—*Effect of buffers and salt on production of acetoin;*[1] *2 to 4 days at 32° C.*

Culture	Proteose-peptone-glucose broth,[2] plus—				Proteose-peptone-glucose broth	
	K_2HPO_4	Na_2HPO_4	$(NH_4)_2HPO_4$	NaCl	With NaCl [3]	Without NaCl
B. alvei No. 683	+++	++++	[4] −	+++	++++	++
B. cereus No. 201	−	±	−	++++	++++	++++
B. cereus var. *mycoides* No. 273	+	+	+	+++	++++	++++
B. subtilis No. 703	++	+++	−	+++	++++	++++

[1] See footnote 1, table 4.
[2] Double-strength broth and 1-percent solutions of the salts were autoclaved separately and combined in equal quantities.
[3] 0.5 percent NaCl added to broth before sterilization.
[4] Some strains of *B. alvei* gave positive results.

LENGTH AND TEMPERATURE OF INCUBATION

Vaughn, Mitchell, and Levine (*110*), working with the *Escherichia coli–Aerobacter aerogenes* group, found that a greater number of cultures produced acetylmethylcarbinol if the temperature of incubation was 30° C. instead of 37°, as prescribed in the standard methods (*1*). These results were confirmed by Snieszko and Skorka (*98*), who also found that 28° was good. Since some strains of *Bacillus cereus* (and var. *mycoides*) grow poorly and some do not grow at all at 37°, it was necessary to test the effect of incubation temperatures.

Tubes of proteose-peptone glucose solution were incubated at 28°, 32°, and 37° C. for 2, 4, and 6 days. The results given in table 6 show that considerable variation appeared in the production of acetoin by *B. cereus* and its var. *mycoides*. A temperature of 37° apparently was too high and 28° rather low, the best being 32°. An incubation period of 4 days was sufficient for these particular strains, but this did not hold for all strains or for other species. By testing a large number of strains of all the species having a positive V-P reaction, it was found that 32° was suitable for all. Certain species (*B. subtilis* group) are not so particular as to temperature, producing acetoin at 32° or 37° about equally, more slowly at 28°. Most of them were positive in 2 days. Considering the genus as a whole, it is not possible to make a single test, for instance, at 4 days, because those organisms developing slowly might be negative. On the other hand, if a longer period is chosen, those growing rapidly might have formed the acetoin and used it up, thus giving a negative test.

There seems to be no uniformity among strains of a species or between species, either as to rate of formation or disappearance of the acetoin. In some cases it did not seem to decrease in quantity even at 20 days' incubation. Usually several tubes were inoculated with a strain, and if a positive test was obtained at any one incubation period the culture held for the next longer period was also analyzed as a

TABLE 6.—*Influence of temperature and length of incubation on production of acetoin* [1]

Culture	Temperature at—								
	28° C. for—			32° C. for—			37° C. for—		
	2 days	4 days	6 days	2 days	4 days	6 days	2 days	4 days	6 days
B. cereus:									
No. 303	++	++	++	++	+++	++	−	−	+
No. 319C [2]	+	+	±	+	+++	++++	+	±	±
No. 402	+	++++	++++	+++	+++	++++	++	++++	+++
Intermediate No. 248	+	++	++	+	+++	−	+++	+	−
B. cereus var. *mycoides:*									
No. 273	±	+++	++++	+++	++++	++++	−	−	−
No. 324	±	+++	++	−	++++	+++	−	++++	++++

[1] See footnote 1, table 4.
[2] Dissociant of *B. cereus* var. *mycoides* No. 319.

control. A few cultures have been found in which the V-P test was not positive until 10 to 20 days. A persistent negative test was found only in two strains of *B. subtilis* and in one of *B. pumilus.*

KIND OF PEPTONE

In the recommended methods (*1*, *99*) it is stated specifically that proteose-peptone should be used. Burton and Rettger (*12*) tested out Difco, Witte, Eimer & Amend, and Armour peptones and a meat extract for the V-P test on *Aerobacter aerogenes.* They found Witte's to be the best of the peptones, whereas meat extract increased the utilization of sugar. Using a phosphate meat extract glucose medium for the V-P test, they considered it more reliable than the methyl red test. Dorner and Hellinger (*31*) found the following peptones suitable for acetoin production: Witte's, Difco's Bacto-proteose and neo-peptone, Fairchild's, and Merck's. These were used, however, with a meat-broth base. Others also have tested various peptones. To see if the kind of peptone would influence the production of acetoin in the present study, broths were prepared containing, respectively, Witte's peptone, Difco's proteose, tryptone, Bacto- and neo-peptones, and beef extract. Other brands of peptone were not available.

Bacillus cereus (and var. *mycoides*) and *B. alvei* were consistently positive on proteose and neo-peptones and were erratic or negative on the other mediums. *B. subtilis* and its varieties *niger* and *aterrimus*, *B. pumilus*, and *B. polymyxa* were positive on Bacto-peptone as well. Tryptone and Witte's peptones and meat extract were generally inferior. The standard procedure recommending proteose-peptone is confirmed. An amendment that neo-peptone is equally satisfactory for these bacteria may be added.

Of many other mediums that have been recommended for the V-P test (*12*, *47*, *51*, *65*, *85*, *115*), only the following were tried in this work: Fumarate broth, sucrose-peptone-salt broth, and maltose broth. Although the fumarate and maltose mediums were entirely unsatisfactory, the sucrose medium was satisfactory if the organism tested

utilized that sugar. Since many do not, this medium cannot be generally adopted. Two percent glucose was also used and was found to be inferior to the 1 percent generally recommended, thus confirming the results of O'Meara (*85*). The explanation may, perhaps, be found in the recent work of Stahly and Werkman (*101*). They found that with rapid fermentation of the glucose, the redox potential dropped and the glycol accumulated. After most of the glucose has been attacked the Eh rises and the glycol is oxidized to acetoin.

Reaction of Medium

Burton and Rettger (*12*) found that the initial acidity of the medium (containing buffer) had no appreciable effect on the terminal acidity. This fact was tested in the present work, using the unbuffered broth as given above at an initial pH of 8.0, 7.0, and 6.0. The results of the previous investigators were confirmed; namely, that the initial pH had no appreciable effect on the terminal or intermediate acidity. Acetoin formation also was not affected.

Aeration

The effect of aeration had to be considered along with the other factors studied. O'Meara (*85*) used 5 ml. of broth in ¾-inch test tubes because "increased aeration favors the production of acetoin." This fact has been noticed by many others. For instance, Reynolds and Werkman (*87*) reported that vigorous aeration of glucose broth cultures of *Escherichia coli* resulted in the formation of acetoin, and Mickelson and Werkman (*75*) found that aeration under pressure increased the production of acetylmethylcarbinol by *Aerobacter indologenes*. Owing to the difficulties of keeping cultures aerated under pressure, different degrees of aeration were obtained in this work by putting 5 ml. of the modified broth into test tubes of different diameters as follows:

Small tubes, diameter 15 mm.; depth of liquid approximately 35 mm.
Regular tubes, diameter 18 mm.; depth of liquid approximately 25 mm.
Large tubes, diameter 22 mm.; depth of liquid approximately 18 mm.

A few tests were made using a more shallow layer in 250-ml. Erlenmeyer flasks, but since these gave about the same results as the large tubes and the flasks were awkward to handle, they were not used further.

Before adding the reagents to develop the color in the V-P reaction, the broth culture was poured into medium-sized tubes to avoid any effect that the various-sized tubes might have upon the development of the color. In every case, using many different strains of *Bacillus cereus* and *B. subtilis*, the cultures incubated in the largest tubes gave the strongest reaction for acetoin. Furthermore, a positive reaction was obtained in a shorter incubation period than in the case of the smaller tubes. The cultures in the smallest tubes (15 mm.) were decidedly poor. Certain strains (*B. cereus* var. *mycoides* No. 936 and *B. subtilis* var. *aterrimus* No. 740) that were definitely positive in the large tubes were either negative or doubtful in the small tubes. On the other hand, *B. polymyxa* and *B. alvei* produced acetoin equally as well in the small as in the larger tubes, although usually not so rapidly.

Test Reagents for Acetoin

Attempts too numerous to mention have been made to increase the delicacy or hasten the V-P reaction, and there seems to be no method that has been accepted above all others. It was only natural, therefore, that a few of the more common methods should have been compared in this work. In the first tests, the KOH-copper-sulfate-ammonia method (*99*) was used and gave good results. Later this method was compared with the alpha-naphthol method of Barritt (*3*) and the creatine method of O'Meara (*85*). Previously, Vaughn, Mitchell, and Levine (*111*) had compared the KOH, the alpha-naphthol, and the creatine methods and strongly recommended the alpha-naphthol. Unfortunately, they modified O'Meara's reagent by dissolving 0.3 percent creatine in the KOH before using, whereas O'Meara added the dry powder to the test solution.

These two creatine methods (*85*, *111*) were thoroughly tested on the cultures under observation. When fresh, the 0.3 percent creatine in NaOH gave about as good results as O'Meara's method, but after aging a few days it became weaker. After standing a month its strength was so reduced that a 4+ reaction by O'Meara's method would be only 1+ or a plus-minus by the modified reagent.

The alpha-naphthol method was more difficult to read than O'Meara's or the copper sulfate methods, contrary to the findings of Barritt (*4*). Since the completion of this work, Batty-Smith (*5*) has given a good review of recent literature on the detection of acetoin and on the basis of his tests on *Aerobacter aerogenes* he has recommended a combination of the alpha-naphthol, creatine, and ferric chloride reagents. It is said to be more sensitive. Incidentally, he used a medium containing a phosphate and an incubation temperature of 37° C. for 2 days, relying upon the sensitivity of the method rather than on the improvement of growth conditions for the maximum production of acetoin by the organism.

In the writers' laboratory O'Meara's method is entirely satisfactory: Merely add an equal quantity of 40 percent NaOH to the incubated broth, jar a few milligrams of creatine from a knife point on to the surface, shake, and read within 1 hour. As the broth does not contain a phosphate, its color is very slight and there is no interference with the bright red color shown in the positive V-P reaction.

Conclusions on Production of Acetylmethylcarbinol

From the results obtained it is concluded that species of the genus *Bacillus* should be grown in a broth containing 7.0 gm. of proteose-peptone, 5.0 gm. glucose, and 5.0 gm. sodium chloride for the production of acetylmethylcarbinol. The medium should be dispensed 5 ml. per large test tube (18 mm. in diameter), incubated at a temperature of 32° C. for 2, 4, 6, 10, and 20 days, and the V-P test made by the creatine or a similar sensitive method.

The formation of acetylmethylcarbinol is one of the most reliable characters of this group and may be used in the separation of certain species.

STORAGE OF FAT

In the writers' preliminary classification of this group (*95*) the occurrence of fat globules in the rods grown on glucose agar was used to separate *Bacillus megatherium* and *B. cereus* from the others. After 3 or 4 days' incubation at 28° C. the cells showed large fat globules when stained in a wet mount with Sudan III (saturated alcoholic solution). These globules were pink or light orange and were not in so sharp a contrast with the rest of the cell as was desired. Nile-blue sulfate (*22, p. 80*) and oil red O (*22, p. 44*) were tried but were found to be inferior to Sudan III. Sudan IV gave slightly better results, because less precipitate was formed in the wet mount.

Hartman in 1940 (*48*) introduced Sudan black B as a stain of bacterial fat. It had been used previously by Lison (*70*) to stain fat in tissues, because it proved to be superior to a large number of fat stains, including Sudan III, and is absolutely specific for fat. Hartman recommended a saturated solution of Sudan black B in ethyleneglycol because there was no evaporation from the wet mount, consequently no streaming, and the mount might be examined at one's leisure. The fat droplets were stained dark blue or black and stood out in good contrast.

Using Hartman's method the writers were surprised to find that all the members of this genus stored fat. Most strains of *B. megatherium* and *B. cereus* formed numerous large globules, although certain other strains of these species formed fewer and smaller globules. In strains of *B. megatherium* containing only small droplets it was very hard to decide with Sudan III whether they were fat-positive or negative. There was no doubt, however, when Hartman's method was used. *B. brevis*, which had been suspected of being fat-positive when stained with Sudan III, was clearly positive with Sudan black B. Rods of *B. subtilis* and its two varieties *aterrimus* and *niger*, *B. firmus*, members of the *B. circulans* group, and the round-spored group contained a few small globules that could be seen easily with the black stain but were not visible with the Sudan III.

Recently Burdon and others (*11*) have recommended making a suspension of the organisms in 0.5 ml. of a saturated alcoholic solution of Sudan black B, spreading a drop on a slide, drying, and counterstaining with safranin. They stated, "even the most minute fat droplets are brilliantly revealed." In the fat-negative group they placed *B. mesentericus* (probably the American strain which is *B. pumilus*), *B. subtilis* (Ford strain), *B. subtilis* (Marburg strain), and *B. vulgatus*. Using Hartman's method, the writers have found these bacilli to be definitely fat-positive, as much so as *B. circulans*, which Burdon considered fat-positive.

The advantage of a permanent-stained smear by Burdon's method is more than offset by the disadvantage of spherical particles of precipitated stain occurring above or below a rod, thus making a decision difficult as to whether it is really a fat globule or an artifact. By using the wet mount no difficulty along this line was found.

The production of fat by the genus is, therefore, quantitative rather than qualitative. This applies not only to different species but to

different strains within the species. Obviously this character must be used with discretion and not as a definite criterion on which to separate certain species from others.

In place of the presence of fat the writers selected a criterion used by Gibson and Topping (*43*) to differentiate *B. megatherium* and *B. cereus* from the rest of the bacilli. The normal rods of these two species cultivated on glucose or glycerol-nutrient agar appeared vacuolated or foamy when smeared and stained with a dilute solution of fuchsine, largely because of the many large fat droplets. In the case of a variant and in the absence of large fat globules, the presence of many large swollen unstained forms (shadow-forms) were equally as instructive.

The smaller rods of the remaining species colored evenly, because the fat globules were fewer and much smaller. Shadow-forms were relatively scarce and much smaller. There seemed to be less difficulty in interpreting the dilute fuchsine stain than the fat stain and it is therefore recommended for general use.

FERMENTATION STUDIES

In a preliminary report on the carbohydrate fermentations by members of the genus (*19*), it was concluded that species placed in the same subgroup on the basis of other properties with only a few exceptions attacked the same carbohydrates. It was also observed that a species might vary in its utilization of a single carbohydrate. For example, certain strains of *Bacillus megatherium* readily fermented mannose, whereas others did not. In some cases the mannose-negative cultures upon aging gave rise to daughter colonies that hydrolyzed mannose. Subculturing these colonies produced a mannose-positive strain. It was the purpose of the following experiments to study such variations with other species and to learn, if possible, how much taxonomic reliance could safely be placed on carbohydrate utilization.

It is well known that many of these bacilli, because of their strong proteolytic nature, will not form enough acid from a carbohydrate to overcome the alkalinity produced from organic nitrogen. Consequently, an inorganic nitrogen medium was used as a routine test for determining the availability of a carbohydrate as the only source of carbon. (See section on Methods and Mediums.) Growth and acid formation on the agar slopes proved the ability of the culture to hydrolyze the carbohydrate. A small percentage of the cultures studied was not able to utilize inorganic nitrogen, therefore an organic nitrogen medium had to be used. These particular cultures were not so strongly proteolytic, and acidity could be detected. Examinations were usually made at 3, 7, 14, and 21 days.

Induced Utilization of Sucrose

At the time this special study was begun 109 strains were included in the species *Bacillus cereus*. Of these, 77 were sucrose positive and 32 were negative. All the latter were induced to ferment sucrose by the following procedure. Slopes of the mineral salts agar containing

sucrose and an indicator were inoculated rather heavily from nutrient agar slopes of the sucrose-negative cultures and incubated at 28° C. for 1 month. Then as much as possible of the material on the surface of each slant was again transferred to a slope of fresh medium and incubated another month. Slight growth on the impurities in the agar was obtained even though the sucrose was not attacked. Some of the cultures gave little indication that they would hydrolyze the carbohydrate on the next passage, whereas others showed clearly they were about to do so because of the increased growth. Acid was formed by single colonies or uniformly over the slant, the latter indicating that a number of positive individuals had been brought over on the last transfer. At the end of the second month 23 of the negative cultures had grown well and formed acid from sucrose (table 7). After another passage they fermented the sugar as rapidly as any of the sucrose-positive strains. The rest of the 32 cultures

TABLE 7.—*Utilization of carbohydrates by negative strains induced by monthly serial passage on mineral salts agar containing the specific carbohydrate and the effect on the induced character of storage on nutrient agar*

SUCROSE FERMENTATION

Species	Routine test		Negative strains transferred	Monthly transfers	Acid production	Stored on nutrient agar	Acid production
	Positive strains	Negative strains					
	Number	*Number*	*Number*	*Number*		*Months*	
			17	2	+	11	+
			4	2	+	11	−
			2	2	+		
			4	3	+	10	+
B. cereus	77	32	1	4	+	9	+
			1	6	+	7	−
			1	7	+	6	+
			1	7	+	6	−
			1	7	+		
B. brevis	30	2	1	4	+	10	+
B. laterosporus	0	8	1	9	+	4	−
			8	6	−		

MANNOSE FERMENTATION

			8	2	+		
			5	3	+	6	−
			2	3	+	6	+
B. megatherium	46	25	1	3	+		
			1	4	+	5	+
			1	4	+	5	−
			4	7	+		
			3	7	−		

LACTOSE FERMENTATION

			3	6	+	5	−
B. cereus	5	93	1	6	+	5	+
			1	7	+	3	−
			23	9	−		

ARABINOSE FERMENTATION

B. circulans	31	5	3	4	+	2	+
			1	4	+	2	−
B. cereus	0	123	3	3	−		
B. brevis	0	33	4	7	−		

TABLE 7.—*Utilization of carbohydrates by negative strains induced by monthly serial passage on mineral salts agar containing the specific carbohydrates and the effect on the induced character of storage on nutrient agar*—Continued

XYLOSE FERMENTATION

Species	Routine test		Negative strains transferred	Monthly transfers	Acid production	Stored on nutrient agar	Acid production
	Positive strains	Negative strains					
	Number	*Number*	*Number*	*Number*		*Months*	
B. alvei	0	9	9	6	−		
B. brevis	0	34	1	4	−		
			3	6	−		
B. cereus	0	123	4	9	−		

GALACTOSE FERMENTATION

B. brevis	25	7	3	2	+		
			2	3	+		
			1	4	+		
			1	9	−		

RHAMNOSE FERMENTATION

B. alvei	0	9	9	7	−		
B. megatherium	8	36	1	4	+		
			1	5	+		
			2	11	−		

STARCH FERMENTATION

B. pumilus	0	69	12	7	−		
B. brevis	0	33	3	5	−		
B. laterosporus	0	7	3	5	−		

GLYCEROL FERMENTATION

B. brevis	19	10	2	2	+	10	+
			1	2	+	10	−
			1	3	+	9	+
			3	4	+	8	+
			1	4	+	8	−
			1	10	+		
			1	11	−		

required 3 to 7 monthly transfers on the sucrose agar before they attacked the carbohydrate.

As each one of the cultures became sucrose positive it was carefully examined to eliminate any possibility of contamination. Some of them were then subcultured on nutrient agar and added to the stock collection. After several months they were transferred from the nutrient agar stock to the sucrose inorganic salts medium and observed for acid formation. The results are shown in the last two columns of table 7. Although most of the cultures were still sucrose positive at the end of the storage period, some of them had already reverted and no longer formed acid from the sugar. It will be noted that 17 negative strains became positive after 2 monthly transfers and remained so after 11 months' storage on nutrient agar, whereas 4 others that became positive at the same time became negative again

after the storage period. There seemed to be no relation, therefore, between the length of time required for the development of the ability to use the sucrose and the stability of the character.

The two sucrose negative strains of *B. brevis* were positive after four and nine transfers, respectively. The former was still positive after 10 months, but the latter had become negative again after 4 months. The cultures of *B. laterosporus* were discarded as negative after 6 months, because the initial inoculum dwindled with each transfer and nothing visible was left on the slopes to transfer.

Induced Utilization of Other Carbohydrates

To induce fermentation of mannose, 25 mannose-negative strains of *Bacillus megatherium* were subjected to the same procedure as that for utilization of sucrose. All except 3 became positive after 2 to 7 transfers (table 7). After storage for 5 or 6 months, 6 strains of the 9 tested became negative again. Similar results are shown by *B. brevis* on glycerol.

The fermentation of lactose by *B. cereus* is not common; only 5 of the 98 strains tested were positive by the routine test. Of the negative strains, 31 were transferred serially, resulting in 5 becoming positive in 6 or 7 months. Only 1 of these retained this property after 5 months' storage. Similar results were obtained from *B. megatherium* on rhamnose.

The routine tests using *B. cereus* and *B. brevis* were never positive on arabinose and xylose, nor were any of the 18 strains tested induced to use these carbohydrates. Similar results were obtained with *B. laterosporus* on sucrose, *B. alvei* on xylose and rhamnose, and *B. pumilus*, *B. brevis*, and *B. laterosporus* on starch.

To summarize, a total of 82 cultures were induced to use a carbohydrate that was not fermented previously in the routine test. Of the 57 cultures kept under observation, 20 (30 percent) lost this ability after storage on nutrient agar from 2 to 11 months at room temperature. The nature of these "trained" enzymes and the interesting problems connected with them must remain for future study. They do not seem to fulfill Dubos' specifications of an adaptive enzyme (*32*), nor were they apparently as stable as the lactase of *Escherichia coli-mutabile* described by Lewis (*68*). They seem, however, to be much like certain enzymes of several types of the dysentery bacilli reported by Courmont and Rochaix (*24*, *25*). After repeated transfer on a medium containing the particular substance, their strains would ferment a carbohydrate that was normally not attacked. This new fermentation capacity frequently proved to be only temporary.

Value of Fermentation Tests

Fermentation tests on members of the genus *Bacillus* have a taxonomic value, provided they are made on a medium containing ammonium phosphate as the source of nitrogen and if they are interpreted liberally. There is no doubt of their importance in cases where all strains are either positive or negative, but where various percentages are positive a problem is presented. It has been found that if a majority of the cultures are positive, most of the others may be in-

duced to utilize that carbohydrate, although this acquired character may not be stable. On the other hand, if only a small percentage of the strains are positive, the chances of inducing the utilization of a carbohydrate by such negative cultures are not very good. In this case it is recommended that the few positive strains be called biotypes.

In cases where inorganic nitrogen is not utilized by the organism, organic nitrogen will have to be used and allowances made for any ammonia that may be formed that might neutralize the acid produced.

AGGLUTINATION RESPONSES

The serology of the members of the genus *Bacillus* was investigated to a very limited extent, because facilities for such work were available to one of the writers (F. E. Clark) for only a short time. For these studies young healthy rabbits were immunized in successive lots of three to five animals each against the whole cultures selected as antigens. Eight to ten injections, varying from 1.0 ml. of nephelometer turbidity 2 to 3.0 ml. of nephelometer turbidity 3 of phenolized antigen (0.3 percent) given at approximately half-weekly intervals, produced satisfactory serum titers. Agglutination tests were performed with young whole cultures harvested from nutrient agar surfaces.

The agglutination responses of seven strains of *B. alvei*, four strains of *B. circulans*, and five of *B. laterosporus* to antiserums for two strains of *B. alvei* and one of *B. laterosporus* are given in table 8. The three antiserums agglutinated only strains of their homologous species and with one exception (No. 686) they agglutinated all those strains. Three strains of *B. laterosporus* bore the name *B. orpheus* when added to the writers' collection. Because of their

TABLE 8.—*Serologic agglutination responses of Bacillus alvei, B. circulans, and B. laterosporus*

Agglutinogens	Agglutinins [1]		
	B. alvei		*B. laterosporus* No. 681
	No. 662	No. 685	
B. alvei:			
No. 662	320	160	–
No. 683	160	160	–
No. 684	640	320	–
B. para-alvei: [2]			
No. 685	320	640	–
No. 686	–	320	–
Isolations: [3]			
No. K	320	320	–
No. M	320	160	–
B. circulans:			
No. 676	–	–	–
No. 677	–	–	–
No. 678	–	–	–
No. 679	–	–	–
B. laterosporus:			
No. 314			1,280
No. 340			640
B. orpheus: [2]			
No. 661			80
No. 681	–	–	640
No. 682			640

[1] Reciprocal of highest serum dilution giving positive agglutination.
[2] Name under which the cultures were received.
[3] Discarded.

morphological and biochemical similarity they have been combined in one species in this work. The serological results are further evidence of the synonomy of *B. laterosporus* and *B. orpheus*.

It is interesting that *B. circulans* did not cross agglutinate with either of the *B. alvei* antiserums. These species are closely related but can be separated on physiological grounds, as shown below.

Bacillus subtilis, *B. vulgatus*, *B. niger*, and *B. aterrimus* have been grouped in one species, i.e., *B. subtilis*, in this work because of failure to find some character other than pigmentation and type of growth that might separate them. Details are given above in the section on dissociation of *B. aterrimus* and *B. niger*, and below in the description of *B. subtilis*.

Antiserums were developed against *B. subtilis* Nos. 231 and 238 (formerly called *B. vulgatus*), *B. subtilis* var. *aterrimus* No. 230, and *B. subtilis* var. *niger* No. 254. Agglutination responses by a number of strains of *B. subtilis* and its varieties to the four antiserums are presented in table 9. The results offer no basis for the separation

TABLE 9.—*Serologic agglutination responses of Bacillus subtilis and its varieties*

Agglutinogens	Agglutinins [1]			
	B. subtilis		*B. subtilis* var. *aterrimus* No. 230	*B. subtilis* var. *niger* No. 254
	No. 231	No. 238		
B. subtilis:				
No. 231	10,240	80	80	80
No. 242	—	—	640	—
B. vulgatus: [2]				
No. 237	320	640	160	160
No. 238	40	10,240	80	80
B. aterrimus: [2]				
No. 330	40	10,240	80	160
B. subtilis* var. *aterrimus:				
No. 230	—	—	10,240	40
No. 259	80	80	10,240	—
No. 260	160	80	10,240	40
No. 261	160	80	5,120	40
No. 262	5,120	80	160	80
No. 274	—	—	2,560	—
No. 275	80	—	5,120	—
No. 276	40	40	2,560	—
B. subtilis* var. *niger:				
No. 219	160	80	—	320
No. 220	40	40	40	2,560
No. 223	160	160	—	320
No. 224	80	320	80	1,280
No. 225	160	80	80	640
No. 254	—	40	—	2,560
No. 265	320	320	160	640

[1] Reciprocal of highest serum dilution giving positive agglutination.
[2] Former name.

of the four former species, i.e., *B. subtilis*, *B. vulgatus*, *B. aterrimus*, and *B. niger*. The responses tend to be strain or variety specific with cross agglutination occurring at lower titers.

Since this work was done, Lamanna (*59*) has separated *B. subtilis* from *B. vulgatus* by using precipitinogens obtained from spores by boiling in HCL and by observing whether there was transverse splitting when spores germinated. Although this separated two strains of *B. subtilis* (S8 and 102) that originally came from Ford, he discredited culture 102 because it came through more than one hand, whereas culture S8 came from Ford through Soule. Unfortunately,

the history of strain 102 was not given correctly. It came from Ford through the American Museum of Natural History to one of the present writers (N. R. Smith) in 1923, was given to the American Type Culture Collection in December 1941, and relayed to Lamanna at once. The explanation of any difference in the two strains is not immediately ascertainable. It does seem, however, that Lamanna should have recognized the factor of dissociation and should have used more cultures before drawing conclusions.

Fourteen antiserums were prepared against strains of *B. brevis*. Although satisfactory homologous titers were given by seven antiserums (*B. brevis* Nos. 604, 605, 616, 628, 632, 641, and 664) cross-agglutination responses were not observed and these were discarded. The seven remaining antiserums (*B. brevis* Nos. 603, 611, 612, 614, 622, 635, and 640) cross-agglutinated only 1 or 2 strains (table 10).

The agglutination reactions of *B. brevis* were, therefore, taxonomically unsatisfactory because the antiserums were too specific.

TABLE 10.—*Summary of cross-agglutination responses of Bacillus brevis and B. laterosporus*

Agglutinogens	Agglutinins [1]								
	B. brevis							*B. laterosporus*	
	No. 603	No. 611	No. 612	No. 614	No. 622	No. 635	No. 640	No. 314	No. 661
B. brevis:									
No. 603	5,120	–	–	–	–	5,120	–	–	–
No. 611	–	5,120	–	–	–	–	20,480	–	–
No. 612	–	–	20,480	20,480	20,480	–	–	–	–
No. 614	–	–	20,480	20,480	20,480	–	–	–	–
No. 622	–	–	20,480	20,480	20,480	–	–	–	–
No. 635	5,120	–	–	–	–	5,120	–	–	–
No. 640	–	2,560	–	–	–	–	20,480	–	–
B. laterosporus:									
No. 314	–	–	–	–	–	–	–	5,120	10,240
No. 661	–	–	–	–	–	–	–	5,120	10,240

[1] Reciprocals of highest serum dilutions giving complete agglutination.

The two strains of *B. laterosporus* included in the same table did not react with any of the *B. brevis* antiserums. This is remarkable because of the close relationship of *B. laterosporus* to *B. brevis*.

Antiserums effective against one or more strains of *B. brevis* failed to agglutinate other species of sporeformers that were morphologically and culturally distinct from *B. brevis*. For instance, antiserums of *B. brevis* Nos. 604, 612, 616, 640, and 628 failed to agglutinate strains of *B. megatherium*, *B. cereus*, *B. subtilis*, *B. subtilis* var. *niger*, *B. subtilis* var. *aterrimus*, *B. pumilus*, *B. alvei*, *B. polymyxa*, and *B. macerans*.

To determine whether the homologous and heterologous agglutinogens within a cross-agglutination group showed similar or dissimilar agglutinin-absorption capacity, serum against *B. brevis* No. 622 was treated with an excess of the following whole-culture antigens: *B. brevis* Nos. 612, 614, 622, and 632, the latter having failed to cross agglutinate. The unabsorbed serum showed an agglutinin titer of 20,480 for the first three cultures and a negative one for strain No. 632. The antiserum prepared with culture No. 622 and exposed to excessively turbid cell suspensions of strain No. 612 for 30 minutes

gave positive agglutination of cultures Nos. 612, 614, and 622 at dilutions of 1 to 20 and 1 to 200, and negative agglutination at 1 to 2,000 and 1 to 20,000. After the second exposure, positive agglutination at 1-to-10 dilution was observed; and after a third exposure, no agglutination. Similar titer reductions were shown after absorption with either culture No. 614 or culture No. 622. The serum against *B. brevis* No. 622 exposed to *B. brevis* No. 632 still retained a titer of 20,480 for cultures Nos. 612, 614, and 622.

The usefulness of serology in the taxonomy of this genus is uncertain. It is especially so since McCoy's (*73*) able demonstration of positive and negative responses among descendants of a single culture. Other serological studies noted in the literature are of little value because of the prevalence of misnamed strains and the confusion of identities that has existed between the American and European *B. mesentericus* and between *B. cereus* and *B. subtilis*. Since very few workers have included in their reports any morphological or physiological descriptions the reader cannot be sure that their cultures were correctly named.

A review of the positive serological reactions recorded by several workers who used vegetative cells as antigen discloses a few responses apparently between strains of separate species. Carbone and others (*13*) studied the agglutination of 26 strains by 13 antiserums prepared with bacilli of the *B. mesentericus* group. Although they concluded that most of the species were antigenically distinct, *B. aterrimus*, *B. mesentericus*, *B. fuscus*, *B. tyrosinogenes*, and *B. carotarum* were regarded as a subgroup because of their coagglutination at high titer. Their strains of *B. mesentericus fuscus* were of the European *B. mesentericus*, which is considered here as identical with *B. subtilis*. *B. aterrimus* and *B. tyrosinogenes* were found by the writers to be so closely related to the same species that they were classified as *B. subtilis* var. *aterrimus*. Carbone's first 3 responses, therefore, were probably within 1 species. In the present work, however, *B. carotarum* proved to be in synonymy with *B. megatherium*. If Carbone's culture was authentic, strains of two distinct species were antigenically similar. Sievers and Zetterberg (*93*) observed positive complement fixation between strains of the following separate species: *B. mycoides* antiserum with *B. vulgatus* (*B. subtilis*), *B. cereus* antiserum with *B. vulgatus*, *B. subtilis* antiserum with *B. cereus* and *B. mycoides*, and *B. vulgatus* antiserum with *B. mycoides*. *B. cereus* antiserum also reacted with *B. subtilis* by the precipitation test.

The agglutination reactions of *B. macerans* and *B. polymyxa*, the gas-forming facultative bacilli, were investigated by Porter, McCleskey, and Levine (*86*). All 16 strains of *B. macerans* were agglutinated by the 2 *B. macerans* serums tested. Although each of 9 *B. polymyxa* serums reacted with its specific strain and a few others, no one antiserum agglutinated all 71 cultures of *B. polymyxa*. There was no reaction between the serum of one species and the cells of the other.

Lamanna (*57*, *58*) and others have sought to avoid the complex antigen of the vegetative cell by using the serological reactions of the spores. Lamanna found that the spores of 10 strains of *B. cereus*

and 1 of *B. cereus* var. *mycoides* were agglutinated at low titer by the spore antiserum of *B. agri* (probably *B. pumilus*). With the exception of the spore antiserum of *B. cereus*, his remaining antiserums are believed by the writers to be too specific for identifying a well-represented species. The agglutination of the spores of 31 cultures of *B. cereus*, including several of the variety *mycoides*, by the spore antiserum of 1 *B. cereus* strain was especially interesting to the writers who disagree with Lamanna's division of *B. cereus*, as represented by the 31 strains on the basis of the fermentation of starch, glycerol, and sucrose. (See description of *B. cereus*, p. 47.) It is regrettable that this antiserum was not tested against many more strains of *B. cereus* and of *B. cereus–B. megatherium* intermediates, for it gave good promise of being specific for the species.

Although the results of the serological studies of the present work are meager, they point to a similarity between the serology and bacteriophagy of the bacilli. It was not unusual to find that a bacteriophage developed against a strain of a particular species lysed only that one strain; or that a second bacteriophage for another culture of the same species dissolved several strains; or that a third bacteriophage prepared with still another culture was active against all the strains of the particular species. No cultural or biochemical differences could be found between the strains sensitive and resistant to the first and second bacteriophages. Not every bacteriophage isolated, therefore, could be used taxonomically.

The antiserums of the few bacilli studied by the authors resembled the bacteriophages in their reactions with the strains of each homologous species. The agglutinins for *B. brevis* No. 604, for example, reacted only with culture No. 604; serum against *B. subtilis* agglutinated part but not all of the cultures of *B. subtilis;* and the antiserum of *B. alvei* No. 685 agglutinated all the strains of *B. alvei.* Although this apparent similarity between the bacteriophagy and serology of the bacilli may or may not have a real basis, the possibility that the understanding of the underlying principles of one may provide understanding of the other should not be overlooked.

BACTERIOPHAGY

Bacteriophage was used during the first part of this study for the separation of certain species. In the case of four species it was very helpful at a time when knowledge of the cultures was meager and the reliability of most reactions uncertain. As acquaintance with these species increased, other reactions were found that were as dependable as the bacteriophage and much simpler in application. Although sensitivity or resistance to a strain of bacteriophage has not been included in the final definition of any of the species, the methods and results of the experiments are given here for those interested in bacteriophage as a taxonomic tool.

Each strain of bacteriophage was isolated from soil. About 10 gm. of garden soil were added to 100 cc. of a 16-hr. nutrient broth culture of the organism against which a bacteriophage was desired. After incubation of 2 to 3 days at room temperature the mixture was filtered through paper and then through an L2 Pasteur-Chamberland filter. Ten cc. of an 8-hr. culture of the same organism was

added to the filtrate and held at 28° C. overnight. Ordinarily it was necessary to repeat the filtration and the addition of a young culture only once or twice before the reinoculated filtrate appeared clear and limpid after its overnight incubation. Upon this first evidence of bacteriophage a few drops of the lytic culture were thoroughly mixed with 5 cc. of a 6- to 8-hr. culture of the sensitive bacillus, and 2 to 3 drops of the mixture were spread over the surface of an agar plate. If bacteriophage was present, the resulting growth was punctured with lysed areas or plaques. A small inoculating loop was touched to a single well-isolated plaque and then dipped into a tube of another young broth culture. After this culture was dissolved the quantity and the potency of the lytic agent were increased by combining it with larger and larger numbers of the susceptible strain. If the lytic culture became cloudy with secondary growth it was filtered before any further addition of the sensitive organism. The strength of the bacteriophage was roughly determined by serial dilution in tubes containing 10 cc. of sterile, nutrient broth. One cc. of a 16-hr. culture of the bacillus against which the bacteriophage had been developed was pipetted into each of the dilution tubes. If clearing was observed in 24 hours in the 10-million or 100-million dilution, the lytic filtrate was considered potent enough for testing unknown strains.

Susceptibility of unidentified bacilli to a bacteriophage was determined in the following way: The strain to be tested was inoculated into a tube of nutrient broth and incubated for 24 hours. Then one drop of the 24-hr. culture was placed into each of two more tubes of broth; 1 cc. of the active filtrate was pipetted into one; and the other served as a control. The tubes were held at room temperature and observed at 4, 8, 24, and, occasionally, 48 hours. A cleared bacteriophage tube and a turbid control were considered evidence of lysis. If the phage tube remained cloudy a loopful of its contents was streaked on a nutrient agar slope. Definite, unmistakable plaques in the growth were also regarded as proof that the bacillus was sensitive to the bacteriophage. No result was accepted until it had been checked at least once by repeating the procedure just described.

TABLE 11.—*Specificity of strains of bacteriophage for Bacillus megatherium*

Cultures of *B. megatherium*	Type of growth	Lysis by bacteriophage [1] for culture—		
		No. 240	No. 258	No. 239
No. 234	White, smooth	−	−	+
No. 239	Yellow, smooth	−	−	+
No. 240	White, smooth	+	+	+
No. 241	Cream, smooth	−	−	+
No. 245	White, smooth	+	+	+
No. 257 [2]	White, intermediate	+	+	+
No. 258 [2]	Yellow, rough	+	+	+
No. 268	Cream, rough	−	−	+
No. 269	Cream, smooth	+	+	+
No. 270	Yellow, rough	−	−	+
No. 271	Yellow, intermediate	−	−	+
No. 283	White, smooth	+	+	+
No. 284	-----do	+	+	+
No. 285	-----do	−	−	+

[1] + indicates lysis; −, no visible lysis.
[2] Rough stages of No. 234.

Not all strains of bacteriophage can be used taxonomically, because too many are specific for one culture or a few cultures of a species. Only the bacteriophage that lyses a great majority of the strains of a species has any taxonomic value. In the attempt to isolate a lytic agent for identifying *Bacillus megatherium* the first two developed against strains Nos. 240 and 258 were too limited in their effect (table 11). Their lytic actions were identical but had no relation to the pigment of the cultures tested, to their stage of growth, or to any other property as far as could be determined. The bacteriophage developed against No. 239, on the other hand, lysed all the strains included in the table. Subsequent trials showed that it lysed 83 percent of all the strains of *B. megatherium* and 16 percent of the *B. megatherium-B. cereus* intermediates.

Too great a specificity was also shown by the three bacteriophages developed against *B. laterosporus* and none was isolated capable of attacking all the strains of this species (table 12). Again, no other

TABLE 12.—*Specificity of strains of bacteriophage for Bacillus laterosporus*

Cultures of *B. laterosporus*	Lysis by bacteriophage [1] for culture—		
	No. 661	No. 314	No. 347
No. 314	−	+	+
No. 340	−	+	+
No. 347	+	+	+
No. 590	−	+	+
No. 661	+	−	−
No. 681	+	−	−
No. 682	+	−	−

[1] See footnote 1, table 11.

difference was recognizable between the susceptible and the resistant strains.

For the isolation of a bacteriophage for *B. cereus*, the choice of strain No. 201 was an especially fortunate one. The resulting lytic culture attacked all strains of *B. cereus* except those of the rhizoid variety *mycoides* (table 13).

TABLE 13.—*Effectiveness of bacteriophagy as a taxonomic tool*

Species of *Bacillus* tested	Strains	Lysis by bacteriophage for—			
		B. megatherium No. 239	*B. cereus* No. 201	*B. pumilus* No. 236	*B. brevis* No. 605
	Number	*Percent*	*Percent*	*Percent*	*Percent*
B. megatherium	73	83			
B. megatherium–B. cereus intermediates.	19	16	26		
B. cereus	128		[1] 100		
B. subtilis	162				
B. subtilis–B. pumilus intermediates.	2			100	
B. pumilus	70			100	
B. laterosporus	8				
B. laterosporus–B. brevis intermediates.	1				100
B. brevis	40				90

[1] Variety *mycoides* was resistant, but the nonrhizoid dissociants were lysed.

As shown in the section on the dissociation of *B. mycoides*, these rhizoid strains dissociated to a nonmycoid form identical with *B. cereus* and were susceptible to the bacteriophage. In the present collection all strains of the closely related anthrax bacillus were also lysed.

A bacteriophage against *B. cereus* var. *mycoides* No. 319 was developed that lysed the rhizoid strains, their nonrhizoid variants, and cultures of *B. cereus*. Since it was isolated some time after the bacteriophage for *B. cereus* No. 201, it was not used so extensively as the latter.

A lysogenic culture was also developed that attacked the smooth stage of *B. subtilis* No. 231. At first it lysed only that particular form, but after its potency had been increased it dissolved the rough stage as well. When other strains of *B. subtilis* were tested with this bacteriophage, the smooth ones and a few of the rough ones were susceptible. If a rough, resistant *B. subtilis* was dissociated, the smooth variant was lysed. This bacteriophage, however, was not used in the study of the entire species as represented in the writers' collection, since the volume of work required for the dissociation of the numerous rough strains was prohibitive.

A bacteriophage for *B. pumilus* No. 236 lysed all the strains of *B. pumilus* and three of the four *B. subtilis–B. pumilus* intermediates. None of the rough or smooth strains of *B. subtilis* selected were susceptible.

In addition to the lytic cultures for *B. megatherium* No. 239, *B. cereus* No. 201, and *B. pumilus* No. 236, 1 for *B. brevis* was obtained and found to be especially useful taxonomically. Of the 41 strains of *B. Brevis* and the *B. brevis–B. laterosporus* intermediate, 37 were sensitive to a bacteriophage for *B. brevis* No. 605.

METHODS AND MEDIUMS

Microscopic Examination

The following observations were made on the vegetative rods of cultures incubated 1 to 2 days at 28° C. on nutrient agar:[6] Size of the rods, Gram's reaction, formation of shadow-forms, capsules, and chains. The appearance of the protoplasm of rods grown on glucose-nutrient agar[7] for 2 to 3 days and lightly stained with fuchsine or any other ordinary bacterial dye also was noted. Cells from a glucose-nutrient agar slope also were examined for reserve fat by Hartman's method (*48*). A wet mount was prepared with a saturated solution of Sudan black B in ethylene glycol. Fat droplets in the rods were stained dark blue or black.

The size and shape of the spores and the shape of the sporangium proved to be valuable points for separating the species into three groups. It should be borne in mind, however, that complete reliance on any one character is not to be expected. Some observer might think that the sporangium was definitely swollen, whereas another would

[6] Nutrient agar: Beef extract 3 gm., peptone 5 gm., agar 13 gm., water 1,000 ml. adjusted to pH 6.8.
[7] Glucose-nutrient agar: Nutrient agar plus 0.5 percent glucose.

recognize the fact that that part of the vegetative rod not included in the sporangium had shrunken, giving the false impression of bulging. Familiarity with the three groups would avoid such errors. The number of spores, the time of their formation, and the thickness of the spore wall also were noted. In certain species sporulation on beef-extract agar [8] and peptone agar [9] was compared with sporulation on nutrient agar.

Macroscopic Examination

The macroscopic appearance of the growth on various mediums is, with a few exceptions, too variable to be of aid in the identification of species. Although little dependence was placed on them, the following cultural characters were described: General appearance and size of colonies on nutrient agar, quantity and character of growth on slopes of nutrient agar, on glucose-nutrient agar, on glucose-nitrate agar, in nutrient broth, and on potato. Tyrosine agar, soybean agar, and proteose-peptone acid agar also were used for certain species.

Glucose-nitrate agar was used for the recognition of *Bacillus megatherium* and *B. subtilis*. The formula was as follows: K_2HPO_4 1.0 gm., $NaNO_3$ 1.0 gm., agar 13 gm., water 1,000 ml., and glucose 10 gm. The glucose was added after the agar had been melted. Slants of this medium were inoculated, and the extent of growth and formation of pigment were noted at 3 and 7 days. This agar or any other medium with a fermentable carbohydrate served to demonstrate the black pigment of *B. subtilis* var. *aterrimus*.

Nutrient broth had little value in this study. Its composition was the same as that of nutrient agar without the agar.

Potato plugs were autoclaved with 5 to 10 ml. of distilled water in small screw-capped bottles or potato tubes. The potato cultures were examined at 3 and 14 days, particularly for gas and pigment formation.

Proteose-peptone-acid agar recommended by Stern, Hegarty, and Williams (*105*) was used for *B. coagulans*. It consisted of proteose-peptone 5 gm., yeast extract 5 gm., glucose 5 gm., K_2HPO_4 4 gm., and water 500 ml. The pH was adjusted to 5.0. This was sterilized and mixed with an equal volume of sterile 2-percent agar just before using.

Soybean agar gave a luxuriant growth of several strains of *B. megatherium*, which seemed ill-nourished on nutrient agar. It was prepared by autoclaving 100 gm. of yellow soybeans with 1,000 ml. of distilled water for 1 hour at 20 pounds' pressure. The broth was filtered through paper and made up to volume. Agar was added as usual and pH adjusted to 6.8 if necessary. This medium was introduced by Tsen and Sung (*109*).

Tyrosine agar was used to demonstrate the black pigmentation of *B. subtilis* var. *niger* and, to a less extent, that of *B. megatherium*. It was made by adding 0.05 percent tyrosine to nutrient agar. Pigmentation occurred in 2 to 14 days.

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[8] Beef-extract agar: Beef extract 3 gm., agar 13 gm., water 1,000 ml.
[9] Peptone agar: Peptone 5 gm., agar 13 gm., water 1,000 ml.

Physiological Examination

Casein hydrolysis was demonstrated on milk agar plates prepared by mixing equal quantities of sterile skim milk and sterile 2-percent agar, both cooled to 45° to 50° C. before mixing. After solidifying, cultures were streaked on the plates and observed for growth and clearing of the casein. This milk agar is a modification of Hastings' medium (*49*). It was preferred to milk because of less variability in the results.

Gelatin hydrolysis was determined by a modification of the Frazier technique (*39*). The cultures were streaked on plates of nutrient agar containing 0.4 percent of gelatin. After incubating at 28° C. from 2 to 14 days, depending on the rate of growth, the plates were covered with 8 to 10 ml. of the following test solution: Distilled water 100 ml., concentrated HCl 20 ml., and $HgCl_2$ 15 gm. This reagent formed a white opaque precipitate with the unchanged gelatin and left a clear zone where the gelatin was decomposed. The tendency of some cultures to spread was checked by drying the plates in the 28° incubator for 4 to 6 days before inoculation. This method is to be preferred to the gelatin stab, because incubation temperature need not be low and weak strains that might be negative in the stab will show positive. It is qualitative, whereas the stab is quantitative.

Growth in 4 percent NaCl nutrient broth was used in separating the varieties of *Bacillus sphaericus*. Inoculations were made from a young nutrient broth culture and examined for growth at 3 and 7 days.

The ability to grow at pH 6.0 was determined on slants of nutrient agar. The pH was carefully checked after autoclaving for any change in reaction.

The hydrolysis of uric acid was tested in the medium of Löhnis (*71, p. 96*) modified by the addition of NH_4NO_3. The formula used by the writers was as follows: Uric acid 1 gm., KCl 0.2 gm., $MgSO_4$ 0.2 gm., K_2HPO_4 0.5 gm., NH_4NO_3 1.0 gm., agar 13 gm., and water 1,000 ml.

To determine the maximum temperature of growth, inoculations were made to nutrient agar slants and held for 1 to 3 days in a water bath inside a constant-temperature incubator. The temperature was raised or lowered 2° C. at a time until the highest temperature at which growth occurred was ascertained for each culture.

The reduction of nitrates to nitrites was considered of little value except in the differentiation of *B. subtilis* and *B. pumilus*. In this one instance it was very useful. The test was made on cultures grown in nutrient broth plus 0.1 percent KNO_3 for 3 and 5 days. To 5 ml. of the culture was added 0.5 ml. of a 1-percent solution of potato-starch paste (boiled), 0.5 ml. of a 0.4-percent solution of KI, and, after mixing, 1 drop of concentrated H_2SO_4 (*107*). If nitrites were present a blue color was formed. If there was much nitrite, heavy blue precipitate resulted and gas was evolved. In the case of a negative reaction 5 ml. of the culture was always tested for nitrates by adding a few drops of diphenylamine and pouring concentrated H_2SO_4 down the side of the tube to form a layer underneath. A blue

color at the junction of the two layers was indicative of nitrates (*117*).

The above test method for nitrites was compared with the sulfanilic acid methods recommended in the manual of pure culture study (*99*). Identical results were given by the two methods.

The final pH value of glucose broth culture was found instructive with certain species. The medium contained glucose 5 gm., proteose-peptone 10 gm., and distilled water 1,000 ml. After incubation at 28° C. for 1 week, the pH of each culture was determined colorimetrically.

The production of urease was demonstrated by growing the culture on slopes of nutrient agar and testing for urease after 3 and 7 days. The growth was washed off with 2 ml. of distilled water and divided equally between two clean tubes. A drop of phenol red indicator was added to each and the reaction brought to pH 7.0 by a few drops of very dilute HCl or NaOH. Approximately 0.02 gm. of crystalline urea were mixed with the suspension in one tube and the other was kept as a control. If urease was present the suspension with the urea became very alkaline in a few minutes.

For the reduction of methylene blue a semisolid medium of the following composition was used: Glucose 5 gm., proteose-peptone 10 gm., agar 2.5 gm., methylene blue 0.004 gm., and distilled water 1,000 ml. Tubes 15 mm. in diameter containing 8 ml. were inoculated and examined at 1, 3, 5, 7, 10, 14, and 21 days.

The hydrolysis of starch was determined by streaking plates of nutrient agar containing 1 percent potato starch that had been added after filtering. After incubating from 1 to 8 days, depending on the growth rate of the culture, the plates were flooded with 95 percent alcohol. If the starch remained unchanged the medium became white and opaque, but if it was hydrolyzed a translucent zone appeared around or underneath the growth (*53*). Cultures in the habit of spreading over the entire surface of the agar were inoculated on plates that had been dried in the 28° C. incubator for 4 to 6 days. This permitted a reading of weakly positive results.

The formation of crystalline dextrins from starch was detected by the iodine test of Tilden and Hudson (*106*). The strains were incubated at 37° C. for 2 weeks in 15 ml. of the following medium: Oatmeal 50 gm., $CaCO_3$ 20 gm., and distilled water 1,000 ml. For the test, 0.5 ml. of the clear supernatant from an oatmeal culture was incubated at 40° with 1 ml. of a 3-percent starch solution (Takamine or White Rose). At 15-minute intervals 3 drops of the digest were mixed on a spot plate with a drop of one-tenth normal iodine or Gram's iodine solution. A loopful of the mixture was dried on a clean microscope slide. If crystalline dextrins were present small blue dots (hexagonal crystals at higher magnification) appeared as the blue color of the iodine-starch mixture changed to purple. As the digestion continued the size and numbers of these blue hexagonals increased, and long crystalline needles in fanlike arrangements spread over the area of the drop. It was often possible to find crystalline dextrins in the oatmeal cultures at 2 or 3 days by combining some of the supernatant liquid of the culture with iodine. A positive result

here did away with the long incubation and the digestion of the starch solution at 40°.

For the fermentation studies an ammoniacal nitrogen medium was used, a modification of the one proposed by Ayers, Rupp, and Johnson (*2, p. 13*). The formula was as follows: $NH_4H_2PO_4$ 1.0 gm., KCl 0.2 gm., $MgSO_4$ 0.2 gm., agar 13 gm., and distilled water 1,000 ml. The pH was adjusted to 7.0, and 12 ml. of a 0.04 percent solution of bromcresol purple was added as the indicator. After tubing and autoclaving a solution of the test carbohydrate, sterilized separately by filtration through an L2 Pasteur-Chamberland filter, was pipetted into each tube in such quantity as to result in a 0.5-percent concentration of the carbohydrate. The tubes were incubated overnight, examined for contamination, inoculated, and observed for growth, acid, and gas at intervals of 3, 7, 14, and 21 days.

If a strain failed to hydrolyze glucose in this inorganic nitrogen medium, it was inoculated on slopes of nutrient agar with 0.5 percent glucose and an indicator, usually bromcresol purple, and on control slopes of nutrient agar with the indicator. If acid was formed from the glucose, it indicated that the strain could not use ammoniacal nitrogen, and an organic nitrogen medium had to be used for the tests. Unless otherwise indicated, ammoniacal nitrogen was used in this work.

The evolution of gas from glucose could in certain cases be induced or increased by the addition of metallic iron to glucose broth. Iron filings were heated to redness in a flame and, after proper cooling, were dropped into tubes of sterile nutrient broth with 0.5 percent glucose. Usually 0.25 percent agar was incorporated in the broth to retard the escape of the gas bubbles.

The utilization of citrate was useful in some cases. The cultures were examined for growth and change in reaction on slants of citrate agar of the following composition: Tap water 1,000 ml., agar 13 gm., KH_2PO_4 0.5 gm., NH_4NO_3 2 gm., sodium citrate 2 gm., and 10 ml. of a 0.04 percent phenol red solution. The pH was adjusted to 6.8 before the addition of the indicator. This formula is a modification of Koser's citrate ammonium phosphate solution (*56*).

The medium recommended by the manual (*99*) and standard methods (*1*) for the Voges-Proskauer reaction was modified after experiments had shown that phosphates inhibited the formation of acetoin by some of these organisms. (See section on Factors Affecting the Production of Acetylmethylcarbinol.) The medium used contained 7.0 gm. of proteose-peptone, 5.0 gm. of glucose, 5.0 gm. of NaCl, and 1,000 ml. of distilled water. Five-ml. portions in 18-mm. tubes were inoculated and incubated at 32° C. for 2, 4, 6, 10, and 20 days. The presence of acetylmethylcarbinol was demonstrated by the appearance of a red color after the addition of an equal volume of a 40-percent solution of NaOH and a few milligrams of creatine from a knife point and shaking (*85*).

KEY TO SPECIES OF THE GENUS

The key presented below is based upon the descriptions of cultures of the species studied. Effort was made to bring together species

that are related so that the student may not only identify his culture but may get an idea of the natural relations of members of the genus. At best, keys are artificial and in the case of bacteria they are often misleading. If all bacteria had definite, unalterable characters and fell into distinct aggregates, a key could be made that would always be of value in placing the particular organism. Unfortunately this is not the case. It is the writers' experience, and it seems to be more generally recognized as time goes on, that practically any bacterial character may vary. A key based on single characters, therefore, would be of little value. To obviate this, the key was developed on the basis of two characters as far as possible and closely related species are placed nearby. Should the worker err in the case of one character, the other would prevent the culture from being thrown into the wrong group. For instance, it is quite possible for an investigator to misinterpret his readings in measuring the diameters of vegetative rods, a difference of 0.2 of a micron throwing the culture either into the *Bacillus cereus* or the *B. subtilis* subgroups. The character associated with that of size, i.e., uniform staining, becomes the deciding character in this case.

The key is made for use on normal cultures. Intermediate, aberrant, or weakened strains cannot be allocated by it. They must be studied in detail and then the pattern that their characters develop must be compared with the pattern of known species. Should major differences be found, one would be justified in making a new species. Just what are major differences is a subject on which not many will agree. No rules can be laid down that will apply to all genera or even to all species in a genus. The writers adopted the criterion that a character must be relatively stable in order for it to be used to distinguish a species. Whether it is stable can be shown by studies on dissociation and by observations on a number of strains. Should strains otherwise alike have a character that varies quantitatively and even be negative in some cases, it is a good indication that the character cannot be depended upon. For instance, the production of nitrites from nitrates by *B. cereus* varies from very strong to negative and is consequently considered unreliable. On the other hand, this same character in *B. subtilis* is always positive and has been used as one of the properties separating this species from *B. pumilus*.

Intermediates occur more often between certain species than between others. These have been omitted from the key because of the complications that would arise. They are best shown in the chart showing the numbers and relationships of the cultures studied (fig. 1).

The relative abundance of any one species occurring in the writers' collection probably bears little relation to its occurrence in nature. For instance, *B. subtilis*, represented by 119 strains (fig. 1), would seem to be the most common organism, instead of *B. cereus*. The discrepancy is due to a greater number of cultures bearing other names being assigned to *B. subtilis* than were similarly assigned to *B. cereus* and to a larger number of identifications of unnamed cultures.

Group 1 contains more than 75 percent of the cultures studied and no doubt comprises those species most often found. It might be subdivided into two parts: *B. megatherium* and *B. cereus* in one

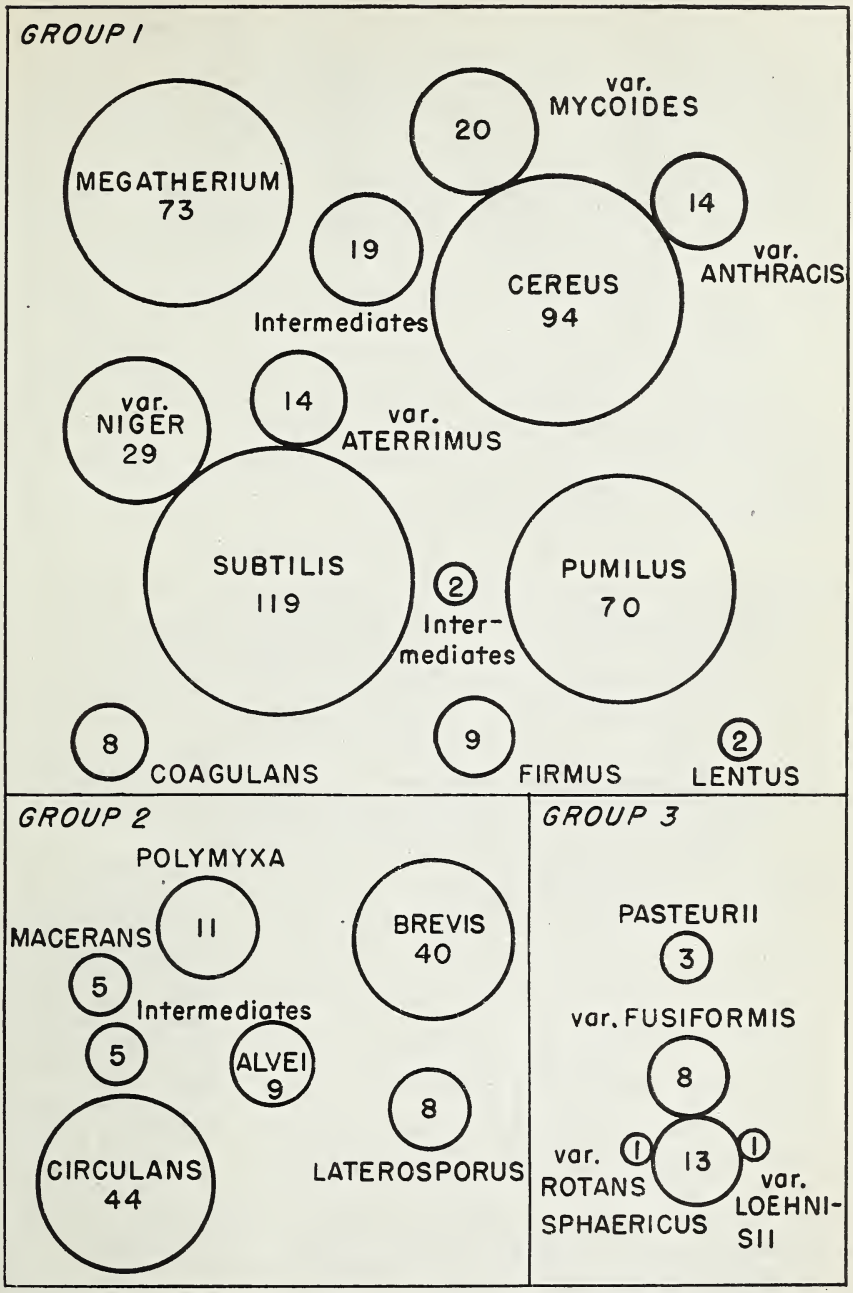

FIGURE 1.—Chart showing the relative numbers and, to some extent, the relationships of the *Bacillus* species studied. The area of each circle equals the number of strains indicated, multiplied by 10 mm.^{2}

and *B. subtilis* and *B. pumilus* in the other. Lamanna did this (*57*, *58*), but erroneously gave the name "small celled species" to the latter group. He apparently was not acquainted with the species in groups 2 and 3, the rods of which are much smaller than *B. subtilis*. Instead of Lamanna's "small celled species" the old designation "hay bacilli" seems to fit this subgroup and certainly does not lead to any confusion as to identities.

Group 2 might also be subdivided, in this case vertically instead of horizontally as in the case of group 1 (fig. 1). *B. circulans* and its relatives are very saccharolytic, whereas *B. brevis* is strongly proteolytic. Morphologically they are indistinguishable and seem to represent a natural group, albeit their activities on sugars and proteins vary a great deal.

It is quite evident that groups 2 and 3 are not adequately represented in this study. Whether they are more or less important in nature than is indicated by the chart cannot be stated. Certainly more cultures should be isolated and studied in order to gain more information concerning the variability of each species. Studies on dissociation are especially needed.

The key to the species studied follows.

GROUP 1. SPORANGIA NOT DEFINITELY SWOLLEN

a. Spores oval to cylindrical, central to terminal; spore wall thin; sporangia only slightly swollen if at all; Gram-positive.
  b. Diameter of the rod 0.9μ or more; protoplasm of the cells grown on glucose or glycerol agar vacuolated if lightly stained.
    c. Acid from arabinose or xylose with ammoniacal nitrogen; acetylmethylcarbinol not produced.....*B. megatherium*.
    cc. No acid from arabinose or xylose; acetylmethlycarbinol produced.....*B. cereus*.
      1. Rhizoid growth.....*B. cereus* var. *mycoides*.
      2. Causative agent of anthrax.....*B. cereus* var. *anthracis*.
  bb. Diameter of vegetative rods less than 0.9μ; protoplasm of cells grown on glucose or glycerol agar stain uniformly.
    c. Growth at pH 6.0; acetylmethylcarbinol produced.
      d. Gelatin hydrolyzed; acid from arabinose or xylose with ammoniacal nitrogen.
        e. Starch hydrolyzed; nitrites produced from nitrates.....*B. subtilis*.
          1. Black pigment on carbohydrate media only.....*B. subtilis* var. *aterrimus*.
          2. Black pigment on tyrosine media only.....*B. subtilis* var. *niger*.
        ee. Starch not hydrolyzed; nitrites not produced from nitrates.....*B. pumilus*.
      dd. Gelatin not hydrolyzed; no acid from arabinose or xylose.....*B. coagulans*.
    cc. No growth at pH 6.0; acetylmethylcarbinol not formed.
      d. Casein hydrolyzed; urease not formed.....*B. firmus*.
      dd. Casein not hydrolyzed; urease produced.....*B. lentus*.

GROUP 2. SPORANGIA DEFINITELY SWOLLEN BY OVAL SPORES

aa. Spores oval, rarely cylindrical, central to terminal; spore wall thick, remnants of sporangium often adhering; sporangia definitely swollen; Gram-variable.
  b. Carbohydrates fermented with acid and gas.
    c. Acetylmethylcarbinol produced; crystalline dextrins not formed from starch.....*B. polymyxa*.
    cc. Acetylmethylcarbinol not produced; crystalline dextrins formed from starch.....*B. macerans*.

bb. Gas not formed from carbohydrates.
c. Starch hydrolyzed.
d. Acid from arabinose and xylose with ammoniacal nitrogen; acetylmethylcarbinol not formed.....*B. circulans.*
dd. No acid from arabinose or xylose; acetylmethylcarbinol produced.....*B. alvei.*
cc. Starch not hydrolyzed.
d. pH of glucose proteose-peptone broth cultures less than 8.0; citrates not utilized.....*B. laterosporus.*
dd. pH of glucose proteose-peptone broth cultures 8.0 or higher · citrates utilized.....*B. brevis.*

GROUP 3. SPORANGIA SWOLLEN BY ROUND SPORES

aaa. Spores spherical, terminal or subterminal; sporangia swollen, usually clavate; carbohydrates not attacked; Gram-variable.
b. Growth on ordinary media; urea or free ammonia not necessary for growth.....*B. sphaericus.*
1. Maximum temperature allowing growth below 37° C.....*B. sphaericus* var. *rotans.*
2. Urease produced.....*B. sphaericus* var. *fusiformis.*
3. No growth at pH 6.0 or below.....*B. sphaericus* var. *loehnisii.*
bb. No growth on ordinary media; urea or free ammonia necessary for growth.....*B. pasteurii.*

DESCRIPTIONS OF SPECIES STUDIED

GROUP 1. SPORANGIA NOT DEFINITELY SWOLLEN

Bacillus megatherium

Bacillus megatherium De Bary, Vergleichende Morph. und Biol. der Pilze, Mycetozoen und Bakterien, p. 499. 1884.

In De Bary's report the name of this organism was spelled "megaterium." It is assumed (*8*) that this was a typographical error and that the name was derived from the two Greek roots, *mega* meaning great and *therium* meaning animal. If so, the name was well chosen, because its vegetative rods are the largest of any of the sporeforming bacteria. In 1883, Zopf (*118, p. 66*) gave the name of *Bacterium tumescens* (regarded here as a synonym) to a similar organism, thus antedating De Bary's report by a year. This reference is usually overlooked and Zopf's publication in 1885 (*119, p. 82*) is cited as the original. Both Zopf's and De Bary's accounts are inadequate to characterize either species. Zopf (*119, p. 82*) regarded them as distinct. Since then, the description of *Bacillus megatherium* has often been emended and has resulted in a clearer definition of the species than in the case of *Bact. tumescens.* The writers recommend, therefore, that *B. megatherium* be retained as nomen conservandum and that the name of *Bact. tumescens* be discarded.

The characters of *B. megatherium* listed below are based upon the study of 73 strains. Of these, 6 were type cultures, 27 bore names in synonymy, 3 bore other names, 13 were received unnamed, and 24 were isolations.

CHARACTERS [10]

**Vegetative rods.*—1.2μ to 1.5μ by 2.0μ to 4.0μ; single or in short chains; ends rounded; stained protoplasm appears granular or foamy; occasional shadow-forms; motile; Gram-positive. *Variations:* [11] 0.9μ to 2.25μ by 1.0μ to 5.0μ; filaments and chains long and tangled; ends squared; protoplasm stains quite evenly; many shadow-forms; nonmotile; budding from end and side of rod; or Gram-variable.

On glucose nutrient agar, rods larger, longer, and more vacuolated than on nutrient agar; fat globules large and numerous. *Variations:* More shadow-forms than on nutrient agar; cells irregular in shape, some with pointed ends, some like corkscrews (wet mount); Gram-variable; or fat globules small and few.

**Sporangia.*—Sporangia not distinctly swollen.

**Spores.*—1.0μ to 1.2μ by 1.5μ to 2.0μ; oval, central, or paracentral; many formed in 48 hours. *Variations:* Diameters 0.8μ to 1.4μ; shapes irregular, reniform, oviform, almost spherical, or cylindrical; lateral; or no spores at 48 hours, a few in 3 to 6 days.

Colonies.—Large; smooth; soft; glistening; round; convex; entire; nonspreading; dense; creamy white to yellow. *Variations:* Rough; somewhat like *B. cereus;* concentrically ridged; or thin-edged.

Nutrient agar slants.—Growth abundant; smooth; soft to butyrous; opaque; glistening; slightly spreading; nonadherent; creamy white to yellow; some browning with age and showing pellucid dots. *Variations:* Rough; somewhat like *Bacillus cereus;* slightly wrinkled; tough; adherent; nonspreading.

Glucose-nutrient agar slants.—Growth usually more abundant and softer (somewhat slimy) than on nutrient agar. *Variations:* Gummy; coarsely wrinkled; pellucid dots more distinct.

**Glucose-nitrate agar slants.*—Growth very heavy; raised.

Potato.—Growth abundant; smooth; soft to slimy; glistening; spreading; creamy white, pale to lemon yellow, or pink. *Variations:* Rough; wrinkled; potato blackened; orange; or no growth.

Soybean agar slants.—Growth abundant with better sporulation and fewer shadow-forms than on nutrient agar. *Variations:* Rough; wrinkled; or sporulation slower than on nutrient agar (two strains).

Tyrosine agar slants.—A few strains form deep black pigment.

Nutrient broth.—Growth medium to heavy and turbidity uniform, with or without abundant sediment; no pellicle. *Variations:* Turbidity flocculent or granular; pellicle thin and friable; or broth clear with flocculent sediment.

**Voges-Proskauer reaction.*—Negative.

Utilization of citrate.—Positive.

**Fermentation tests.*—Acid without gas from arabinose, glucose, fructose, maltose, sucrose, dextrin, inulin, salicin, glycerol, and mannitol; usually acid from xylose, galactose, mannose, and raffinose. Acid variable with lactose; usually no acid with rhamnose.

**Starch hydrolysis.*—Positive.

Nitrites from nitrates.—Variable; majority of strains negative.

[10] Most important characters are starred (*). Unless otherwise stated, nutrient agar is used.

[11] Variations observed are listed singly. One or more of these characters apply to each variant.

Maximum temperatures for growth.—Majority of strains grow at 40° to 45° C. *Variations:* A few failed to grow above 38°. and a few will grow at 48° C.

Hydrolysis of uric acid.—Variable; no correlation with other characters; previously thought to be a distinguishing property of *B. carotarum;* decomposition of uric acid was also demonstrated by certain strains of *B. megatherium.*

Gelatin hydrolysis.—Positive.

Casein hydrolysis.—Positive.

TYPE CULTURES STUDIED [12]

The following type cultures of *Bacillus megatherium* were received and studied in detail.

Bacillus megatherium

No. 234, from AMNH, 1923.
No. 308, from Soule, Mich. Univ., 1936.
No. 892, from Porter, Iowa Univ., 1940; Bredemann; Neide.
No. 893, from Porter, 1940; Edwards; Kral No. 19. Culture very slow and weak in its fermentation of carbohydrates, otherwise typical.
No. 894, from Porter, 1940; Edwards; Conn.
No. 895, from Porter, 1940; Edwards; Novy.

Bacillus megatherium

From ATCC, 1939, No. 71; AMNH No. 734A; Ford. See *B. pumilus* No. 735.
From ATCC, 1939, No. 72; Ford; Kral. See *B. pumilus* No. 736.
From ATCC, 1939, No. 943; Levine No. 2062. See *B. subtilis* No. 737.

SYNONYMY

Named cultures that apparently conform to their original descriptions were identified by the writers as *Bacillus megatherium,* as follows.

Bacillus capri Stapp, Centbl. f. Bakt. [etc.] Abt. 2, 51: 19. 1920.

From Porter:	*B. megatherium* No.
1937; Stapp	607
1940; Bredemann	822
1940; Claussen; Stapp	824

These three cultures agree with each other and, in general, with Stapp's description. In all their properties they are identical with a normal *B. megatherium,* except for their reduction of nitrates to nitrites and in the slightly smaller diameter of their rods. The production of slime on glucose agar noted by Stapp is no greater than that of most strains of *B. megatherium.* Contrary to his observation that no reserve materials were present, fat can be demonstrated in cells of all three strains if grown on glucose-nutrient or glycerol-nutrient agar.

Bacillus carotarum Koch, Bot. Ztg. 46: [277]–287, [325]–332, [341]–350, illus. 1888.

From Porter:	*B. megatherium* No.
1937; Bredemann; Blau strain	608
1940; Stapp; Claussen	828
1940; Claussen	829

[12] Alphabetical abbreviations for the more common sources of cultures are as follows:
AMNH—American Museum of Natural History, New York.
ATCC—American Type Culture Collection, Georgetown University, Washington, D. C.
NCA—National Canners' Association, Washington, D. C.
NCTC—National Collection of Type Cultures, Lister Institute, London.
NIH—National Institute of Health, Bethesda, Md.
USDA—United States Department of Agriculture, Washington, D. C.
USPHS—United States Public Health Service, Washington, D. C.

All the characters of these three strains studied by the writers are identical with those of *B. megatherium*. The presence of cylindrical cells in long threads and single, short, swollen ones described by Koch is not unusual in a slightly rough culture of *B. megatherium*. Although Koch noted the granular appearance of the cell and the similarity in size between his bacillus and De Bary's, he considered the microscopic appearance important enough for a new species.

Bacillus carotarum

From Porter, 1940; Bredemann; Stapp. See *B. circulans* No. 826.
From Porter, 1940; Stapp. See *B. pumilus* No. 827.

Bacillus cobayae Stapp, Centbl. f. Bakt. [etc.] Abt. 2, 51: 10. 1920.

From Porter:	*B. megatherium* No.
1937; Stapp	610
1940; Bredemann	835
1940; Claussen	837

The three cultures of *B. cobayae* are identical. They reduce nitrates to nitrites, as stated by Stapp, but since this is a variable character of *B. megatherium* it cannot be used as a means of differentiation. In fact, the writers do not consider it worthy of varietal status.

Bacillus danicus Löhnis and Westermann, Centbl. f. Bakt. [etc.] Abt. 2, 22: 253. 1908.

From Löhnis, Leipzig:	*B. megatherium* No.
1914, B strain	245
1914	246

These cultures were described as capable of fixing small quantities of nitrogen in mannitol solution. This ability, however, has not been generally accepted, the explanation being that the small gains in nitrogen were due to experimental error.

Bacillus danicus, from Porter, 1937; Glathe. *See B. subtilis* No. 601.

Bacillus graveolens Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 496, 535. 1901.

From Porter:	*B. megatherium* No.
1937; Bredemann	615
1940; Stapp	872

Gottheil stated that the growth of *B. graveolens* was relatively thin and finely wrinkled and not so slimy or so thick as *B. tumescens* (*B. megatherium*). Lawrence and Ford (*61, p. 307*) studied a strain from Kral's collection and found that it had all the cultural reactions of *B. megatherium* with an additional tendency to produce curved or spiral forms. The writers have noticed such forms in other cultures of *B. megatherium* and ascribe them to nutritional factors. The two cultures of *B. gravcolens* above do not differ in any way from *B. megatherium.*

Bacillus graveolens, from ATCC, 1939, No. 7003; Orcutt. See *B. subtilis* No. 730.

Bacillus malabarensis Löhnis and Pillai, Centbl. f. Bakt. [etc.] Abt. 2, 19: 91. 1907.

From Löhnis, Leipzig, 1914 ..... *B. megatherium* No. 239

The culture has been found to be identical with *B. megatherium.* Although *B. malabarensis* was defined as having spindle- or wedge-shaped cells, such cells are frequently observed in cultures of *B. megatherium* and are probably due to unfavorable nutrition. A bacteriophage prepared with this strain lyses 60 of the 73 cultures of *B. megatherium* and some of the *B. megatherium–B. cereus* intermediates.

Bacillus malabarensis

From Porter, 1937; Bredemann. See *B. pumilus* No. 620.
From Porter, 1940; Claussen. See *B. pumilus* No. 891.

Bacillus musculi Stapp, Centbl. f. Bakt. [etc.] Abt. 2, 51: 39. 1920.

From Porter:	*B. megatherium* No.
1937; Stapp	623
1940; Bredemann	907

These cultures reduce nitrates to nitrites, as stated by Stapp, but otherwise cannot be distinguished from a typical *B. megatherium*. (See *B. capri* and *B. cobayae* above.)

Bacillus musculi, from Porter, 1940; Claussen; Stapp. See *B. subtilis* No. 909.

Bacillus oxalaticus (Zopf) Migula, Arb. aus dem Bakt. Inst. der Technol. Hochschule zu Karlsruhe 1: 139–147. 1897.

From Porter:	*B. megatherium* No.
1937; Bredemann	627
1940; Claussen	923

In 1896 Migula received a culture of *B. oxalaticus* from Zopf that had not been described. From Migula's illustrations of the organism, it is apparent that the original was a strain of *B. megatherium* because of the large size of the rods and their vacuolated appearance.

Bacillus oxalactis, from Porter, 1940; Stapp; believed to be a misspelling of *B. oxalaticus*. See *B. cereus* No. 922.

Bacillus petasites Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 535. 1901.

	B. megatherium No.
From ATCC, 1937, No. 89; AMNH No. 735; Ford No. 13	343
From Porter, 1940; Stapp	931

The original description of *B. petasites* indicates a yellow strain of *B. megatherium*. Lawrence and Ford (*61, p. 305*) obtained a culture from Kral and also isolated several from soil and dust. They concluded that these cultures differed from *B. megatherium* only in their yellow pigment. Since most of the cultures of *B. megatherium* used in the present work produce some pigment varying from cream to yellow to pink to brown or black on some medium or other, pigment formation is considered too unstable a property of this species to characterize a variety.

Bacillus petasites

From NCTC, 1937, No. 2606; Ford No. 20. See *B. subtilis* No. 338.
From Porter, 1940; Bredemann. See *B. subtilis* No. 930.
From Porter, 1940; Claussen. See *B. subtilis* No. 933.

Bacillus ruminatus Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 485. 1901.

From Porter:	*B. megatherium* No.
1940; Stapp	951
1940; Claussen	952

The large diameter of the rods (1.4μ to 1.5μ), the formation of fat, and the character of the growth originally ascribed to *B. ruminatus* are characteristics of *B. megatherium*. Laubach and others (*60, p. 499*) stated that it resembled *B. megatherium* closely, except for its porcelain-white growth. Cultures Nos. 951 and 952 show a yellow pigment on potato and agree with *B. megatherium* in all respects.

Bacillus ruminatus

From ATCC, 1936, No. 98: AMNH No. 793; Ford. See *B. pumilus* No. 345.
From Porter, 1937; Bredemann. See *B. subtilis* No. 634.

Bacillus silvaticus Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 32. 1904.

From Porter:	*B. megatherium* No.
1940; Stapp	957
1940; Claussen	958

Neide stated that the rods were 1.2μ to 1.6μ by 4μ and contained vacuoles and fat globules. The spores averaged 1.1μ by 1.7μ and before germination swelled more in width than in length. Growth on nutrient agar was creamy white and on potato it was heavy, yellowish, and slimy. Lehmann, Neumann, and Breed (*63, p. 611*) placed this organism close to *B. megatherium*. Cultures No. 957 and 958 are identical with this and are not at variance with Neide's account.

Bacillus silvaticus, from Porter, 1937; Bredemann. See *B. subtilis* No. 636.

Bacillus (*Bacterium*) *tumescens* Zopf, Die Spaltpilze, p. 66. 1883.

From Porter:	*B. megatherium* No.
1940; Stapp	991
1940; Edwards; Winslow	994
1940; Claussen	995

The history of *B. tumescens* is discussed under *B. megatherium*. The three strains above proved to be typical for *B. megatherium*. They are probably authentic.

Bacillus tumescens

From Porter, 1940; Bredemann. See *B. subtilis* No. 989.
From Porter, 1940; NCTC No. 2607; Ford No. 25A. See *B. pumilus* No. 990.
From Porter, 1940; Edwards; Kral No. 23 smooth and No. 23 rough. See *B. cereus* Nos. 992 and 993.

ASSIGNMENT OF OTHER NAMED CULTURES

The cultures listed and assigned to *Bacillus megatherium* group below do not conform to their original descriptions or are not recognizable because of the inadequacy of their characterizations. They were studied and identified as *B. megatherium*.

Bacillus aterrimus

From Schooley, Bucknell Univ., 1936 ..... *B. megatherium* No. 267

For authentic cultures, see *Bacillus subtilis* var. *aterrimus*.

Bacillius immobilis Steinhaus, Jour. Bact. 42: 783. 1941.

From Steinhaus, USPHS, 1942, No. 81 ..... *B. megatherium* No. 1024

This culture was recovered by Steinhaus from a tube broken while moving his laboratory. He questioned whether it was the original culture. Evidently it was not, because culture No. 1024 is entirely different from his description of it, being a normal *B. megatherium* except for the production of a darker pigment on tyrosine agar and potato.

Bacillus simplex Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 685. 1901.

From Porter, 1940; Edwards; Conn. ..... *B. megatherium* No. 960

Gottheil's description is inadequate, and the emending by Lawrence and Ford (*61, p. 310*) did not help very much. The above strain is a normal, rough *B. megatherium*. On the other hand, four more strains labeled *B. simplex* Nos. 335, 346, 961, and 962 are intermediate between *B. megatherium* and *B. cereus*. See *B. megatherium–B. cereus* intermediates.

IDENTIFICATION OF UNNAMED CULTURES

The following 13 unnamed cultures were received during the course of the work and were identified as *Bacillus megatherium*.

Unnamed cultures

From Meier, USDA, 1937, from air over the Caribbean, *B. megatherium* Nos. 13H, 13M, 15B, 16B, and 19A 690–694
From Reid, Pa. State Col., 1937, from fermenting tobacco, Nos. A1, D1, and H1 695–697
From Porges, Independ. Farm Labs., Ames, Iowa. 1940 771
From Steinhaus, USPHS, 1942, from insects, Nos. 16, 20, and 82 ..... 1021, 1022, and 1025
From ATCC, 1942; Hollweg, Fort George Wright Hosp., Wash. 1026

The authors isolated from soil and identified as *B. megatherium* the following 24 strains: 240, 241, 268–271, 283–289, 360–367, 392, 393, and 753.

Bacillus megatherium–Bacillus cereus intermediates

The occurrence of intermediates between two established species has presented problems of classification in various genera, the present genus being no exception. In some cases the intermediates may be looked upon as weakened or aberrant strains, i.e., they may have lost some original characters because of unfavorable conditions or they may have developed a property not usually attributed to the species.

In the present work there are 19 strains that seem to be intermediate between *Bacillus megatherium* and *B. cereus*. Using the V-P reaction, the utilization of mannitol and the pentoses, growth on glucose-nitrate agar, and susceptibility to the homologous bacteriophage, they can be separated into 4 groups. It is recognized that the basis for some of the separations is very weak and probably subject to variation. Such a grouping, however, is convenient and shows the relation of the intermediates to each other as well as the progression of characters leading from *B. megatherium* to *B. cereus* (table 14).

TABLE 14.—*Bacillus megatherium–B. cereus intermediates grouped according to certain characters and compared with typical strains of those two species*

Designation	Strains	V–P reaction	Mannitol fermented	Pentoses fermented	Growth on glucose nitrate	Strains lysed by phage for—	
						B. megatherium	*B. cereus*
	Number					*Number*	*Number*
B. megatherium	73	−	+	+	[1]++++	60	0
Group A	9	−	+	−	±	[2]1	0
Group B	4	−	−	−	++++	2	1
Group C	5	−	−	−	±	0	3
Group D	1	+	−	+	++++	0	1
B. cereus	94	+	−	−	[3]±	0	94

[1] ++++ indicates abundant growth; +, poor growth; ±, very scant growth.
[2] 4 of the 9 strains were not tested with phage of *B. megatherium*.
[3] 5 strains grew well.

The members of group A do not utilize the pentoses nor will they grow well upon glucose-nitrate agar. The named cultures included here resemble *B. megatherium* more than *B. cereus*, but the isolations are more like *B. cereus*.

Bacillus agrestis Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 468. 1933.

From Porter, 1937; Bredemann
.....*B. megatherium–B. cereus* intermediate No. 602

The morphology and the few physiological tests reported in the original description apply to No. 602. It was lysed by the *B. megatherium* phage.

Bacillus cohaerens Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 689. 1901.

	B. megatherium–B. cereus intermediate No.
From Porter:	
1940; Bredemann	838B
1940; Stapp	839B

There has been considerable confusion over the identity of *B. cohaerens*. Gottheil stated that his organism was 1.1μ in diameter, of varying lengths, and, as nearly as can be judged, was similar to a rough *B. megatherium*. Chester (*15, p. 84*) believed it to be identical with *B. simplex*. Lawrence and Ford (*61, p. 308*) disagreed with Chester, because the culture that they obtained from Kral had rods 0.37μ to 0.65μ in diameter and spores 0.5μ to 0.56μ by 0.93μ to 1.25μ. The growth was thin, soft, spreading, whitish, becoming yellow. This same culture apparently was obtained by the writers from the NCTC. (See below.) It proved to be a typical *B. pumilus* (No. 334) and quite unlike Nos. 838B and 839B.

Cultures No. 838 and 839 of *B. cohaerens* were mixed when received. From each was obtained a small rod (*B. circulans* No. 838A and 839A, respectively) and a larger rod similar to *B. megatherium* (Nos. 838B and 839B, respectively). The measurements of the latter and the similarity of the two cultures to *B. simplex* confirm Chester's observations. Neither of them was lysed by the phage for *B. megatherium* or by the one for *B. cereus*.

Bacillus cohaerens, from NCTC, 1937, No. 2596; Ford 10A; Kral. See *B. pumilus* No. 334.

Bacillus flexus Batchelor, Jour. Bact. 4: 32. 1919.

From Henry, Wash. [State]
Univ. .....*B. megatherium–B. cereus* intermediate No. 665

This culture substantiates Batchelor's opinion that it resembled *B. megatherium* microscopically. Since there is nothing in the original account that disagrees with the present culture, it is undoubtedly authentic. It was not lysed by either of the two phages.

Bacillus niger

From Cameron, NCA, 1937; Breed; Levine,
No. E. 17 .....*B. megatherium–B. cereus* intermediate No. 656

This culture is definitely misnamed. For an account of *B. niger* see *B. subtilis* var. *niger.* It was not lysed by either phage.

Four isolations from soil (Nos. 756, 757, 758, and 759) also belong to group A. Tests for lysis by the *B. megatherium* phage were not made: no lysis occurred with the *B. cereus* phage.

The members of group B fail to utilize mannitol in addition to failing on the pentoses. They grow well, however, on the glucose-nitrate agar slants.

Bacillus simplex

From Porter, 1940; Claussen:
Blau .....*B. megatherium–B. cereus* intermediate No. 962

This strain is lysed by the *B. cereus* phage, which differentiates it from three other strains of *B. simplex* placed in group C. For discussion see the following group.

Bacillus sphaericus

From Porter: *B. megatherium–B. cereus* intermediate No.

1940; Stapp 964
1940; Edwards (Ford No. 25); ATCC 965

The two cultures above are clearly misnamed, because they bear no resemblance to *B. sphaericus.*

Bacillus teres Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 161. 1904.

From Porter, 1940; Stapp...*B. megatherium–B. cereus* intermediate No. 986

Lehmann, Neumann, and Breed (*63, p. 605*) place this as one of the synonyms of *B. albolactis,* probably because Neide listed it that way. Neide also gave *B. globigii* as a synonym, which would indicate *B. subtilis.* Culture No. 986 being an intermediate may be the explanation for Neide's uncertainty. It is not susceptible to either *B. megatherium* or the *B. cereus* phage.

Bacillus teres

From Porter, 1937; Bredemann. See *B. pumilus* No. 637.
From Porter, 1940; Claussen. See *B. subtilis* No. 987.

Physiologically, group C is quite similar to *B. cereus,* except for the lack of a positive V-P reaction. Microscopically, they also differ in having long filaments and shadow-forms and are slower to sporulate.

Bacillus simplex Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 685. 1901.

B. megatherium–B. cereus intermediate No.

From NCTC, 1937, No. 2597; Ford 11 335
From ATCC, 1936, No. 99; AMNH No. 737; Ford; Kral 346
From Porter, 1940; NCTC No. 2597; Ford No. 11 961

According to Gottheil the rods measured 0.9μ by 2μ to 3μ, with some long filaments, and the spores 0.83μ by 1.39μ to 2.2μ. Chester (*15, p. 84*) reported that acid was not formed in glucose broth and that alkalinity increased in

lactose and sucrose broths. He thought it was somewhat related to *B. subtilis*. Lawrence and Ford (*61, p. 310*) also studied it, but they found the measurements smaller, 0.56μ to 0.75μ by 3μ to 4.5μ, and that glucose broth was acidified. Many shadow-forms were also noted, as well as the slowness of sporulation. Of the above cultures, No. 346, weak in 1916 (*61, p. 310*), has now lost entirely its ability to form spores, whereas Nos. 335 and 961 (duplicates) form a few spores on soybean agar, none on nutrient agar. All three cultures form acid on glucose with ammoniacal nitrogen and conform to the original measurements of Gottheil. Only No. 961 is susceptible to the *B. cereus* phage.

Bacillus simplex

From ATCC, 1936, No. 4526. See *B. sphaericus* No. 349.
From Porter, 1940; Bredemann. See *B. subtilis* No. 959.
From Porter, 1940; Edwards; Conn. See *B. megatherium* No. 960.

Two unnamed cultures were studied that also belong to group C.

Unnamed cultures	*B. megatherium–B. cereus* intermediate No.
From NIH, 1937	411
From Babers, USDA, 1937	600

So far there is only one culture in group D. In most respects it resembles *B. cereus*, but it ferments xylose and grows well on glucose-nitrate agar slants. It is susceptible to the *B. cereus* phage.

Unnamed culture from Herron, Queen's Col., 1930
.....*B. megatherium–B. cereus* intermediate No. 248

Bacillus cereus

Bacillus cereus Frankland and Frankland, Roy. Soc. London, Phil. Trans. Ser. B. 173: 279–281. 1887.

Bacillus cereus, one of the first organisms to be described, is widely distributed and outnumbers any other member of the genus found in the soil. In spite of this, it appears to be the least known by bacteriologists, for it has been and is still being isolated and described again and again under a host of names. The account by Frankland and Frankland, although an early one, is adequate and recognizable. A partial excuse for the bacteriologists' lack of familiarity with *B. cereus* may be the mistaken identity of *B. subtilis*, with which it was confused for some time. Conn (*21*) carefully studied the literature and concluded that the name *B. subtilis* has been applied to two different bacilli, a comparatively large-celled organism, 1.0μ or more in diameter with large spores, and to a smaller one, 0.7μ to 0.8μ in diameter with small spores. The former is *B. cereus* (*B. subtilis*, Michigan strain) and the latter the true *B. subtilis*. Bacteriologists should become acquainted with *B. cereus* and its varieties so that it may be recognized when isolated from soil, water, milk, blood, and similar sources.

Bacillus cereus is an excellent example of a "parent species." It has given rise to several variants and to two varieties. The mycoid variety (*B. mycoides*) is basically similar to *B. cereus* and upon dissociation cannot be distinguished from it. The pathogenic variety (*B. anthracis*), which is discussed later, is also so closely related that nonpathogenic strains cannot be separated from *B. cereus* or from dissociates of *B. cereus* var. *mycoides*. The writers have put more time upon the study of these relationships than will appear to the reader and are quite confident that the arrangements shown represent a natural grouping.

The following description of *B. cereus* is based upon 94 strains of which 2 were type cultures, 33 bore names in synonymy, 20 bore other names, 25 were received without names, and 14 were isolated.

CHARACTERS

**Vegetative rods.*—1.0μ to 1.2μ by 3.0μ to 5.0μ; cells usually in short to long tangled chains; ends square; stained protoplasm granular or foamy; no shadow-forms; nonencapsulated; motile; Gram-positive. *Variations:* 0.9μ to 1.3μ by 2.0μ to 6.0μ; filaments; ends rounded; protoplasm stained uniformly or encapsulated; or very rough strains nonmotile; one strain Gram-variable.

On glucose-nutrient agar, cells are larger and more vacuolated than on nutrient agar and contain many large fat globules. *Variations:* Fewer, smaller fat globules, but always vacuolated when lightly stained.

**Sporangia.*—Spores do not swell sporangia appreciably.

**Spores.*—1.0μ by 1.5μ; oval; central or paracentral; many formed in 18 to 24 hours. *Variations:* 0.5μ to 1.2μ by 1.3μ to 2.5μ; few or none formed in 48 hours.

**Colonies.*—Large; usually rough; flat; irregular; whitish, with characteristic mottled appearance by transmitted light described by different writers as resembling ground glass, moire silk, or galvanized iron, due to the parallel, wavy arrangement of the chains of cells; all stages occur from the thin, spreading, very rough and arborescent to the smooth, dense morphotype of colony; borders of the smoother colonies generally mottled and have whiplike outgrowths.

Nutrient agar slants.—Growth abundant; whitish; spreading; dense; opaque or mottled, depending on the morphotype; growth sometimes extends into medium. *Variations:* A few strains form a greenish-yellow fluorescent pigment.

Glucose-nutrient agar slants. — Growth abundant, heavier, and softer than on nutrient agar. *Variations:* Fluorescent strains do not show so much pigment as on nutrient agar.

Glucose-nitrate agar slants.—Scant or no growth. *Variations:* Five strains grow well.

Potato.—Growth abundant; thick; spreading; soft; creamy-white, sometimes with pinkish tinge. *Variations:* Growth restricted; thin; folded; dry; slimy; water slimy; potato darkened; or orange-colored.

Nutrient broth.—Heavy, uniform turbidity with soft, easily dispersed sediment; may or may not have soft, ring pellicle. *Variations:* Flocculent growth, or compact pellicle.

**Voges-Proskauer reaction.*—Positive.

Utilization of citrate.—Usually positive.

Fermentation tests.—Acid without gas from glucose, fructose, maltose, dextrin, and glycerol; usually positive on sucrose and salicin; variable on galactose; negative on xylose, arabinose, rhamnose, raffinose, inulin, and mannitol; usually negative on mannose and lactose. The fermentation of lactose was first considered unusual enough to warrant the separation of the positive strains into variety *albolactis*. This plan was abandoned after several negative strains

were induced to hydrolyze lactose by serial monthly transfers on lactose agar. (See section on Fermentation Studies.)

**Starch hydrolysis.*—Positive. Although some starch negative cultures of *B. cereus* were described by Lamanna (*58*), the writers have found all strains positive by the method of Kellerman and McBeth (*53*). A few of Lamanna's negative strains sent to the writers by Knaysi were also positive in their hands.

Nitrites from nitrates.—Positive. *Variations:* Fourteen strains negative. This agrees with results obtained by Conn and Breed (*23, p. 273.*)

Maximum temperature for growth.—45° C. *Variations:* A few strains failed to grow above 35°; others grew at 48°.

Gelatin hydrolysis.—Positive; rapid.

Casein hydrolysis.—Positive; rapid.

TYPE CULTURES STUDIED

The following authentic cultures of *Bacillus cereus* were received and studied:

Bacillus cereus

No. 232, from AMNH, 1923, No. 724; Ford.
One of five strains of *B. cereus* that grow well on glucose-nitrate agar.

No. 305, from Soule, Mich. Univ., 1936.

SYNONYMY

Thirty-three named cultures that apparently conform to their original descriptions were identified by the writers as *Bacillus cereus* as follows:

Bacillus albolactis (Loeffler) Migula, Syst. Bakt., p. 577. 1900.
B. lactis albus Loeffler, Berlin Klin. Wchnschr. 24, p. 630. 1887.

	B. cereus No.
From ATCC, 1939, No. 7004	721
From Porter, 1940; NCTC No. 2601; Ford No. 15	808

Lawrence and Ford (*61, p. 287*) stated that *B. albolactis* resembled *B. cereus* morphologically, and it fermented lactose. Their identification was probably correct, for Migula noted that *B. albolactis* was similar to the anthrax bacillus.

Strain No. 721 ferments lactose readily. Acid was not formed from lactose by culture No. 808 when it was received. After six monthly transfers on mineral salts-lactose agar the culture grew moderately well and produced acid in a few days. (See section on Fermentation Studies.)

Bacillus cereus fluorescens Laubach, Jour. Bact. 1: 508. 1916.

From Porter, 1937; NCTC No. 2600; Ford No. 14 ..... *B. cereus* No. 645

This culture is morphologically and physiologically like *B. cereus*, except that a greenish-yellow fluorescence is usually shown on nutrient agar, milk agar, and gelatin, as originally stated. Nitrates are not reduced to nitrites.

Bacillus ellenbachensis (Stutzer and Hartleb) Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 540. 1901.
B. ellenbachensis alpha Stutzer and Hartleb, Centbl. f. Bakt. [etc.] Abt. 2, 4: 31. 1898.

From Porter:	*B. cereus* No.
1940; Bredemann; Gottheil	847
1940; Bredemann	848
1940; Stapp	849
1940; Claussen; Kläser	850
1940; Claussen	851

This species has generally been considered the same as *B. cereus*. These results confirm that belief.

Bacillus goniosporus Burchard, Arb. aus dem Bakt. Inst. der Technol. Hochschule zu Karlsruhe 2: 14. 1898.

From Knaysi, Cornell Univ., 1940 ..... *B. cereus* No. 767

Gottheil (*46*) regarded this as identical with *B. ellenbachensis*.

Bacillus lacticola (Migula) Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 168. 1904.
B. lactis V Flügge, Ztschr. f. Hyg. u. Infektionskrank. 17: 299. 1894.
Bact. lacticola Migula, Syst. de Bakt., p. 305. 1900.

	B. cereus No.
From Porter, 1937; Bredemann	617
From ATCC, 1939, No. 4342; Stewart	731
From Porter, 1940; Stapp	877

These cultures are in agreement with the morphology, macroscopic appearance, and the few physiological properties given in Neide's description.

Bacillus lacticola, from Porter, 1940; Claussen. See *B. pumilus* No. 878.

Bacillus lactis (Flügge) Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 337. 1904.
B. lactis I Flügge, Ztschr. f. Hyg. w. Infektionskrank. 17: 272–342. 1894.

From Porter:	*B. cereus* No.
1937; Bredemann	618
1940; Bredemann; Neide	879
1940; Stapp	880
1940; Claussen	881

In 1894 Flügge (*36, p. 294*) described 12 organisms isolated from sour milk. Two years later (*37, p. 208*) he named the first one *B. lactis* I and again defined it as an actively motile, thick, short rod with terminal spores, the colonies of which had branchlike projections and quickly liquefied gelatin. With the exception of the terminal spores these are characters of *B. cereus*. In 1904 Neide described *B. lactis* I more completely and the additional properties also conform to *B. cereus*. Migula in 1900 (*77*) changed the name of Flügge's *B. lactis* I to *B. brevis*. As characterized by Ford (*60, p. 522*) in 1916 and as recognized today, cultures of *B. brevis* differ distinctly from Flügge's and Neide's organism. This situation is discussed under *B. brevis*.

Bacillus metiens Charlton and Levine, Iowa Engin. Expt. Sta. Bul. 132, pp. 18–19. 1937.

From Porter, 1937; Levine ..... *B. cereus* No. 621

This culture, isolated from spoiled ginger ale, was first described in 1927 (*66*), then named and more completely characterized in 1937. From the two descriptions it is recognizable as *B. cereus*. Strain No. 621 grows well on glucose-nitrate agar, otherwise it is typical.

Bacillus petroselini Burchard, Arb. aus dem Bakt. Inst. der Technol. Hochsch. zu Karlsruhe 2: 39. 1898.

From Porter, 1940; Claussen ..... *B. cereus* No. 934

This has generally been considered by taxonomists as identical with *B. cereus*. The culture above substantiates that belief.

Bacillus robur Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 18. 1904.

From Porter:	*B. cereus* No.
1940; Stapp	946
1940; Claussen	947

In describing this species Neide listed *B. cereus* and *B. cursor* as possible synonyms and quoted Gottheil as saying that the latter were probably synonyms of *B. ellenbachensis*. Lehmann, Neumann, and Breed (*63, p. 604*) considered *B. robur* closely related if not identical with *B. cereus*. Cultures Nos. 946 and 947 fail to produce nitrites from nitrates the same as certain other strains of this species (*23*). This character is not taxonomically reliable in this case, and these cultures may be considered authentic.

Bacillus robur, from Porter, 1937; Bredemann. See *B. subtilis* No. 631.

Bacillus sessilis Klein, Centbl. f. Bakt. [etc.] 6: 349, [377]. 1889.

From Knaysi, Cornell Univ., 1940 ..... *B. cereus* No. 768

This organism was found by Klein in the blood of a supposed case of bovine anthrax a few hours after the death of the animal. The cells appeared singly, occasionally in chains; some were motile, others nonmotile. They resembled *B. anthracis*, but the injection of guinea pigs and cattle with the isolate caused no reaction. (See *B. siamensis* and *B. tropicus* below.)

Bacillus siamensis Siribaed, Jour. Infect. Dis. 57: 142–146. 1935.

From Siribaed, Natl. Health Lab., Siam, 1935 ..... *B. cereus* No. 201

Siribaed isolated this strain from a blood culture and reported that it was highly pathogenic to laboratory animals. Clark (*17*) found it to be identical with *B. cereus*. A bacteriophage prepared with this strain lysed all cultures of *B. cereus*, *B. anthracis*, the nonrhizoid dissociants of *B. mycoides*, and some of the *B. cereus–B. megatherium* intermediates.

Bacillus subtilis, Michigan strain, Conn, Jour. Infect. Dis. 46: 341–350. 1930.

	B. cereus No.
From Soule, Mich. Univ., 1936	303
From NIH, 1937, No. 19	537
From NIH, 1937, No. 60. Evans; Conn; Pribram	538
From Porter, 1940; Edwards; Soule	970
From Porter, 1940; Edwards; Novy	976
From Porter, 1940; Edwards; Conn	973
From Porter, 1940; Edwards; Conn and Chester	974

The first five strains above, some of which very likely are duplicates, do not reduce nitrates to nitrites, whereas the last two strains are positive in this respect. Conn and Breed (*23*) found that the ability to reduce nitrates to nitrites was present in the majority of the strains of *B. cereus*, but that some strains otherwise identical with the majority could not form nitrites from nitrates.

Bacillus thuringensis Mattes, Gesell. f. Beförd. Gesemte Naturw., Sitzber. 62: [381]–417. 1927.

From Porter, 1940; Mattes ..... *B. cereus* No. 996

In 1915, Berliner (*7*) described an isolation from the larvae of the flour moth (*Ephestia kuehniella* Zell.) and named it *B. thuringiensis*. Mattes studied the same organism, especially cytologically, but spelled the name *B. thuringensis*, both in the text and in the citation of Berliner's work. Leaving out the letter "i" was no doubt unintentional. These two descriptions and a later one by Chorine (*16, p. 50*) apply to *B. cereus* except for the statement by Chorine that mannitol was fermented, a character absent in *B. cereus* and also in No. 996.

Bacillus tropicus Heaslip, Med. Jour. Austral. 28: 536–540. 1941.

From Heaslip Med. and Vet. Sci. Inst.,
Adelaide, South Australia, 1941 ..... *B. cereus* No. 793

This organism was first found by Heaslip in mice that had been inoculated with the blood of human patients. Its cultural characters were not distinguishable from those of *B. anthracis*. It was pathogenic for mice, guinea pigs, and rabbits, and caused a fever in man. Serologically, it was related to *B. anthracis*, and Heaslip admitted the possibility that it might be variant. No. 793 is an actively motile, normal *B. cereus*. Tests made in 1943 for pathogenicity by the Pathological Laboratory, Bureau of Animal Industry, United States Department of Agriculture, were negative. These results are comparable with Clark's findings on *B. siamensis* (*17*).

Bacillus undulatus Dooren de Jong, Zentbl. f. Bakt. [etc.] Originale (1) 122: 277–286. 1931.

From Porter:	*B. cereus* No.
1940; Dooren de Jong, No. 1110	998
1940; Dooren de Jong, No. 1102	999

The original description of this organism is clearly one of *B. cereus*. Later, Dooren de Jong (*30*) dissociated *B. mycoides* in peptone water and found that the stable S form was the same as *B. undulatus*. He failed, however, to see the similarity of his dissociants to *B. cereus*. (See section on the dissociation of *B. mycoides*.)

ASSIGNMENT OF OTHER NAMED CULTURES

The 20 cultures listed below do not conform to their original descriptions or are not recognizable because of the inadequacy of their characterizations. They were studied in detail and identified as *Bacillus cereus*.

Bacillus aethylicus Fitz, Ber. der Chem. Gesel. 6: 48. 1873; 9: 1348. 1876; 10: 276. 1877.
B. fitzianus Zopf, Die Spaltpilze, p. 52. 1883.

From Porter, 1940; Pribram ..... *B. cereus* No. 804

From the various accounts of this organism it is not possible to judge whether this culture is authentic. It is one of the five strains of *B. cereus* that grow well on glucose-nitrate agar.

Bacillus agri Laubach and Rice, Jour. Bact. 1: 516. 1916.

From ATCC:	*B. cereus* No.
1936, No. 4507; Ford No. 12	310
1936, No. 2; AMNH No. 800; Ford	342

These cultures do not agree with a duplicate of Ford's No. 12 obtained through the NCTC. (See below.) The original account of *B. agri* is too indefinite to determine whether either the ATCC or the NCTC culture is true to type. The name should, therefore, be dropped.

Bacillus agri, from NCTC, 1937, No. 2598; Ford No. 12. See *B. pumilus* No. 336.

Bacillus aterrimus

From ATC:	*B. cereus* No.
1939, No. 944; Levine No. 2025	722
1939, No. 4509; Ford No. 5	723

These two cultures do not form the black pigment characteristic of *B. aterrimus* (see *B. subtilis* var. *aterrimus* below), nor do they agree with it in other respects. Their vegetative rods are slightly smaller than normal; otherwise these cultures are identical with a typical *B. cereus*.

Bacillus coagulans

From Porter, 1940; Bact. Dept., Iowa State Col. ..... *B. cereus* No. 833

For authentic cultures, see *B. coagulans* Hammer.

Bacillus fusiformis

From Porter, 1940; Claussen ..... *B. cereus* No. 867

For authentic cultures of *B. fusiformis*, see *B. sphaericus* var. *fusiformis*.

Bacillus hollandicus

From Porter, 1940; Claussen ..... *B. cereus* No. 875

For authentic cultures of *B. hollandicus*, see *B. brevis*.

Bacillus lactimorbus

From ATCC, 1939, No. 246; Jordan ..... *B. cereus* No. 733

Since this differs radically from the original account (*52, p. 1669*) apparently a mistake has been made. (See *B. sphaericus* No. 732.)

Bacillus lactis niger	*B. cereus* No.
From Gorini, Milan, Italy, 1936, No. 1A	256
From Cameron, NCA, 1937; Breed; Gorini No. 1A	654

These duplicates agree with Gorini's report (*44, 45*) in morphology and macroscopic appearance, but they do not form a black pigment. On the other hand, Gorini's *B. lactis niger* No. 2 does blacken media containing tyrosine but does not agree so well in morphology and appearance. (See *B. subtilis* var. *niger*.) Nos. 256 and 654 ferment lactose readily, two of the few cultures of *B. cereus* that do.

Bacillus laterosporus

From NIH, 1937, Clinical Pathogen series.....*B. cereus* No. 589

For authentic cultures, see *B. laterosporus* in group 2.

Bacillus luteus (Smith and Baker) Garbowski, Centbl. f. Bakt. [etc.] Abt. 2, 19: [641]–655, [731]–749. 1907; 20:99–113. 1907.

From Porter, 1940; Claussen .....*B. cereus* No. 886

Smith and Baker (*97*) stated that this organism resembled *B. mesentericus fuscus* of Flügge in many respects, but that it was larger and formed a bright yellow pigment. Lehmann, Neumann, and Breed (*63, p. 611*) classified it under *B. megatherium*. Another strain studied by the writers proved to be *B. subtilis* (No. 619) and still another *B. pumilus* (No. 885). None of the strains in the collection formed a yellow color. Lacking pertinent data on the original culture, it is not possible to determine the true identity of *B. luteus*.

Bacillus mesentericus ruber

From Porter, 1937; Torrey Res. Sta. .....*B. cereus* No. 644

Descriptions of *B. mesentericus ruber* in no way apply to No. 644. T. Gibson,[13] University of Edinburgh, obtained a culture from very old tubes at the Torrey Research Station that agrees with the early descriptions. He believes that it was at some time accidentally mixed with *B. cereus*. Another culture from Porter, which probably came from Globig, is *B. subtilis* (No. 900). It produces a reddish pigment and resembles the culture that Gibson recovered.

Bacillus mesentericus viscosus

From Porter, 1940; Torrey Res. Sta. .....*B. cereus* No. 903

This culture does not correspond to *B. mesentericus panis viscosus* I or II Vogel. For a discussion of the latter see *B. panis* in the synonymy of *B. subtilis*.

Bacillus oxalactis

From Porter, 1940; Stapp .....*B. cereus* No. 922

This name has not been found in the literature and is probably a misspelling of *B. oxalaticus* Zopf. Migula (*76*) obtained the original culture from Zopf and with it made studies of bacterial structure. He stated that the rods were granular or vacuolated in appearance and measured 2.5μ to 4μ by 4μ to 30μ, a size not even approximated by *B. megatherium*, the largest of this group. The rest of his description provides very little for positive identification. Two other strains of *B. oxalaticus* studied by the writers were identified as *B. megatherium* Nos. 627 and 923. Lehmann, Neumann, and Breed (*63, p. 611*) obtained a culture of *B. oxalaticus* from Kral, the rods of which had a relatively small diameter (0.8μ to 1.6μ). Other characters of their culture resembled the anthrax bacillus. Since No. 922 is also very similar to the anthrax bacillus, it is probably of the same origin and not the original strain, which was probably *B. megatherium*.

Bacillus praussnitzii

From Porter, 1940; NCTC No. 2603; Ford No. 17.....*B. cereus* No. 935

This culture is not rhizoid nor does it ferment lactose, both properties of authentic strains. It may be a dissociant, however. For further discussion, see *B. cereus* var. *mycoides* and the section on Studies on Dissociation.

Bacillus pumilus

From Porter, 1940; Claussen ..... *B. cereus* No. 942

This bears no resemblance to *B. pumilus*.

Bacillus tumescens

From Porter: *B. cereus* No.
1940; Edwards; Kral No. 23 smooth 992
1940; Edwards; Kral No. 23 rough 993

See *B. megatherium* Nos. 991, 994, and 995 for authentic cultures.

[13] Oral communication, Sept. 1939.

IDENTIFICATION OF UNNAMED CULTURES

The following unnamed cultures were studied and identified as *Bacillus cereus.*

Unnamed cultures	*B. cereus* No.
From Herron, Queen's Col., 1930	235
From NIH, 1937, Nos. 8, 10, 14, 16, 29, 35, 41, 66, 77, 80	401–410
The 10 cultures above produce nitrites from nitrates.	
From NIH 1937, Nos. 47–50, 137, 143, 182	539–545
The seven cultures above do not produce nitrites from nitrates.	
From NIH, 1937, from spinal fluid	599
From Haffkine Inst., Bombay, India, 1938	701
From Randall, Georgetown Univ. Med. School, 1939, from blood culture	720
From Mollari, Georgetown Univ. Med. School, 1941, from puncture wound	785
From Mollari, Georgetown Univ. Med. School, 1941, from blood culture	786
From Ross, Reid Memorial Hosp., Richmond, Ind., 1941, from spinal fluid	794
From Allen, Tenn. Univ., 1942, from rayon bath	1006

The following 14 cultures were isolated by the writers and were identified as *B. cereus:* Nos. 202–210, 244, 249, 368–370. These came from soil or air with the exception of No. 202, which came from a blood culture. Nos. 203 and 244 are two of the five strains of *B. cereus* that grow well on glucose-nitrate agar.

Bacillus cereus var. *mycoides*

Bacillus cereus var. *mycoides*, new combination.
B. mycoides Flügge, Die Mikroorganismen, p. 324. 1886.

Physiologically, *Bacillus cereus* var. *mycoides* is identical with *B. cereus.* In growth characteristics it differs remarkably, growing on solid media in long twisted strands, curving and spreading thinly over the surface. In the section on Dissociation it has been shown that this character may easily be lost by growing the rhizoid culture in 100 ml. of nutrient broth, plating after a few days, and selecting colonies. The resulting culture cannot be separated from *B. cereus* and remains stable in its nonrhizoid character, reversion not taking place. *B. mycoides* should, therefore, be considered merely as a variety.

The conclusion above is based upon the study of 4 type cultures, 1 that bore a name in synonymy, and 15 isolations.

TYPE CULTURES STUDIED

The following authentic cultures were studied and are now designated as *Bacillus cereus* var. *mycoides.*

Bacillus mycoides

No. 233, from AMNH, 1923, Ford and Lawrence.
No. 306, from Soule, Mich. Univ., 1936.
No. 911, from Porter, 1940; Edwards; Conn.
No. 912, from Porter, 1940; Claussen.

Bacillus mycoides, from Porter, 1930; Bredemann; Gottheil. See *B. brevis* No. 910.

SYNONYMY

The following named strain conforms to its original description and was identified as *Bacillus cereus* var. *mycoides.*

Bacillus praussnitzii Trevisan, Generi Spec. Batteriacee, p. 20. 1889.
B. ramosus liquefaciens Flügge, Die Mikroorganismen, p. 324. 1886.

From Porter, 1940; Edwards; AMNH.....*B. cereus* var. *mycoides* No. 936

An organism was isolated by Praussnitz in Flügge's laboratory and described by Flügge in 1886 as *B. ramosus liquefaciens* because of its rapid liquefaction of gelatin and the bristly edge of the colonies. It was named *B. praussnitzii* in 1889 by Trevisan. Laubach (*60, p. 495*) stated that *B. praussnitzii* resembled *B. mycoides* in morphology and growth appearance but differed in its active fermentation of lactose. It also liquefied gelatin rapidly, but he did not use this character to separate it from *B. mycoides*. Only one mycoid culture fermenting lactose has been received, and one has been isolated from soil. Both of these when grown in 100 ml. of nutrient broth for several days dissociated to *B. cereus* just as *B. cereus* var. *mycoides* had done. The writers prefer to consider *B. praussnitzii* merely as a lactose-fermenting biotype of the variety *mycoides*.

Bacillus praussnitzii, from Porter, 1940; NCTC 2603; Ford 17. See *B. cereus* No. 935.

ISOLATIONS

Fifteen strains of *Bacillus cereus* var. *mycoides* were isolated from soil and air by the writers and identified: Nos. 273, 317–328, 371, and 372.

Bacillus cereus var. *anthracis*

Bacillus cereus var. *anthracis*, new combination.

B. anthracis Cohn, Beitr. z. Biol. der Pflanz., Heft 2, 1: 177. 1872.

B. anthracis Cohn emend. Koch, Beitrage z. Biol. der Pflanz., Heft 2, 2: 279. 1876.

The literature on *Bacillus anthracis* is extensive and will not be gone into, because it is outside of the scope of the present work. It is in the province of the present writers, however, to call attention of pathologists to the very close relationship existing between the anthrax bacillus and *B. cereus*. Stein (*103*) has recently stated that "pathogenicity constitutes the principal point of difference between typical strains of *B. anthracis* and those of anthrax-like organisms." In the absence of tests for pathogenicity, tests for motility, reduction of methylene blue, hemolysis, fermentation of salicin, rapidity of gelatin liquefaction, action on litmus milk and colony characteristics, when considered as a whole, were of material assistance. Stein used these tests in the negative sense. For instance, an organism that produces marked acidity in salicin broth is not *B. anthracis*. The converse, however, is not true. There are a number of strains of *B. cereus* that do not produce acid from salicin although some may be induced to do so. The other tests, except motility and colony characters, are quantitative in nature and as such are of doubtful value for separating species.

Fourteen type cultures of *B. anthracis* were obtained and examined in the same manner as those of *B. cereus*. After careful consideration of the data, the writers were forced to the conclusion that, aside from its pathogenicity, the only distinguishing character of *B. anthracis* was its nonmotility. Attempts to induce motility by serial transfer in broth failed. Owing to the lack of time, other tests were not made. In spite of this, the writers are not willing to lay much stress upon that character, because certain strains of *B. cereus* and also of its variety *mycoides* have only a few motile cells. Clark (*18*) dissociated a very actively motile strain of *B. alvei* into a nonmotile. It should then be possible to obtain a nonmotile *B. cereus*. Unfortunately, this phase of the work could not be investigated.

Tests for pathogenicity were made in January 1943, on No. 1011 by the Pathological Laboratory, United States Bureau of Animal Industry. It failed to kill guinea pigs and mice. It was reported as differing from the anthrax bacillus in the more rapid action on litmus milk and a slight turbidity in broth. Whether any of the cultures studied by the writers and labeled *B. anthracis* are pathogenic cannot be stated. Much work needs to be done by someone having access to animals, with this question always in mind, namely, If a strain of the anthrax bacillus loses its pathogenicity, is it still the anthrax bacillus or is it *B. cereus?* From the incomplete work of the writers, it would seem that the latter represents the true status of the nonpathogenic anthrax bacillus. If that is true, then taxonomically *B. anthracis* should be merely a pathogenic variety of *B. cereus.* There will probably be objections to such an arrangement from those interested in the diagnosis and control of anthrax, and from a utilitarian standpoint it might be advisable to retain the former designation. But the conclusion is inescapable that *B. cereus* and the anthrax bacillus are very closely related. There seems to be no sharp demarcation between them, as evidenced by the isolation of quasi pathogenic strains that readily lose that character.

TYPE CULTURES STUDIED

The following cultures of *Bacillus anthracis* were studied and are now designated as *B. cereus* var. *anthracis.*

Bacillus anthracis

No. 1007, from ATCC, 1941, No. 10; NIH.
No. 1008, from ATCC, 1941, No. 240; Jordan; NIH.
No. 1009, from ATCC, 1941, No. 241; Jordan; NIH.
No. 1010, from ATCC, 1941, No. 937; Eichorn.
No. 1011, from ATCC, 1941, No. 938; Eichorn.
No. 1012, from ATCC, 1941, No. 4229; Bur. Animal Indus., USDA.
No. 1013, from ATCC, 1941, No. 4728; Bur. Animal Indus., USDA.
No. 1014, from ATCC, 1941, No. 6605; Army Med. School.
No. 1015, from ATCC, 1941, No. 6606; Army Med. School.
No. 1016, from ATCC, 1941, No. 6602.
No. 1017, from ATCC, 1941, No. 6603.
No. 1018, from ATCC, 1941, No. 6604.
No. 1019, from ATCC, 1941, No. 6607.
No. 1020, from Tilley, USDA, 1935.

Bacillus subtilis

Bacillus subtilis Cohn, emend. Prazmowski, Untersuchungen über die Entwickl. und Ferment. einigen Bakterien Arten, Inaug. Diss., Leipzig. 1880.

B. subtilis Cohn, Beitr. z. Biol. der Pflanz., Heft 2, 1: 174; Heft 3, 1: 188. 1875; Heft 2, 2:249. 1876.

There has been a great deal of confusion regarding the identity of *Bacillus subtilis*, largely because of the inadequate early descriptions and the distribution of cultures improperly identified as *B. subtilis* that were in reality the more commonly occurring *B. cereus.* After a careful study Conn (*21*) came to the conclusion that the so-called Marburg strain of *B. subtilis* fitted the early descriptions. His views have been accepted by the International Committee on Bacteriological Nomenclature (*91*).

In the early days of bacteriology nothing was known about the various stages of growth that a species may exhibit—the smooth, rough, mucoid, and dwarf. As early as 1903, however, Chester (*15*) concluded that *B. subtilis* and *B. vulgatus* (Flügge) Trevisan (*108*) were identical, because the cultural characters upon which their separation depended were too variable. His leadership was not followed by Lawrence and Ford (*61, p. 292*) who stated that the two species could be separated by "careful observation of the cultural reactions, particularly in broth and on potato." Recently Lamanna (*57, 59*) has attempted a separation by means of serological tests and by the equatorial germination of the spore with or without splitting along the transverse axis. This divided his cultures of the 2 species into a group of 4 and a group of 12. As the type culture for *B. subtilis* in the group of 4, he chose one of the 2 cultures that came from Ford and placed with *B. vulgatus* the other Ford culture, 2 Marburg strains of *B. subtilis* (including the type culture of the genus), and various other authentic cultures of *B. subtilis*. It would seem very doubtful whether other bacteriologists will agree with Lamanna's conclusions. In view of the variations in other characters obtained by the writers in this work, it does not seem likely that those properties selected by Lamanna would be immutable. It is unfortunate that he did not have a greater number of strains to study and that this phase could not be taken up by the writers.

In the section on dissociation of *B. aterrimus* and *B. niger* it has been shown that the black pigment produced by those organisms can be varied through gray, pink, and to cream color, the latter being that of *B. subtilis*. In addition to this information, stock cultures of *B. subtilis* were tested on potato in 1937 for pigmentation and retested in 1941. Certain strains that were creamy white to brown at first, later were pink; some of those that were pink changed to yellowish or brownish.

Whether the growth of *B. subtilis* is flat, warty, folded, adherent or nonadherent, dry, moist, smooth, or mucoid depends upon the stage of growth. Most of these stages are represented in the literature by named species, as will be seen from the cultures listed under synonomy.

The following description of *B. subtilis* is based upon a study of 119 strains, of which 14 were type cultures, 26 bore names in synonymy, 33 bore other names, 43 were unnamed, and 3 were isolations.

CHARACTERS

**Vegetative rods.*—0.7μ to 0.8μ by 2.0μ to 3.0μ, single or in chains; ends rounded; uniformly stained; no capsules; no shadow-forms; motile; Gram-positive. *Variations:* 0.6μ to 1.0μ by 1.3μ to 6.0μ to filaments; long chains; encapsulated; shadow-forms; a very few strains Gram-variable; a few nonmotile; or budding from end of rod.

On glucose-nutrient agar, rods are the same as on nutrient agar and contain a few small fat globules. *Variations:* Larger cells; more filaments and longer chains; encapsulated; or Gram-variable.

**Sporangia.*—Very little swelling, if any, by spores; those with cylindrical spores often resemble bipolar stained rods.

**Spores.*—0.6μ to 0.9μ by 1.0μ to 1.5μ; oval to cylindrical; central

or paracentral; many formed in 48 hours. *Variations:* 0.5μ to 1.0μ by 1.0μ to 2.0μ; or few formed in 15 to 21 days.

Colonies.—Rough; opaque; dull; adherent; slightly spreading; brownish white to light brown with aging. *Variations:* Smooth to slimy; soft; thin; translucent; dendroid; nonadherent; or creamy white to yellow to orange.

Nutrient agar slants.—Growth abundant; rough; opaque; dull; adherent; slightly spreading; cream-colored to light brown. *Variations:* Smooth; slimy; thin; translucent; dendroid; nonadherent; or yellow to orange; some strains show a greenish fluorescence when grown at 45° C.

**Glucose-nutrient agar slants.*—Growth heavier than on nutrient agar.

**Glucose-nitrate agar slants.*—Abundant growth. *Variations:* Scant growth by seven strains.

**Tyrosine agar slants.*—Same as on nutrient agar.

**Potato.*—Growth heavy; wrinkled to coarsely folded; spreading pellicle over water; off-white to yellow to pink to brown, often brown with aging. *Variations:* Slimy; soft; thin; or warty.

Nutrient broth.—Broth clear with heavy, wrinkled, dry, tough pellicle. *Variations:* Flocculent turbidity with or without pellicle; or greasy-appearing pellicle.

**Voges-Proskauer reaction.*—Positive. *Variations:* Two strains negative.

Utilization of citrate.—Positive. *Variations:* 11 strains negative.

**Fermentation tests.*—Acid without gas on xylose, arabinose, glucose, fructose, galactose, mannose, maltose, sucrose, salicin, glycerol, and mannitol; usually positive on dextrin; variable on rhamnose, raffinose, and inulin; usually negative on lactose.

**Starch hydrolysis.*—Positive.

**Nitrites from nitrates.*—Positive.

Maximum temperatures for growth.—Most strains grow at 50° to 54° C. *Variations:* A few failed to grow above 37° C., and a few will grow at 58°.

**Gelatin hydrolysis.*—Positive.

Casein hydrolysis.—Positive.

TYPE CULTURES STUDIED

The following 14 authentic cultures of *Bacillus subtilis* were received and studied.

Bacillus subtilis

No. 231, from Kellerman, USDA, 1912.
No. 243, from AMNH, 1923, No. 723; Ford.
No. 304, from Soule, Mich. Univ., 1936; Ford.
No. 560, from Bengtson, NIH, 1937.
No. 743, from ATCC, 1939, No. 465. Acetylmethylcarbinol production very weak and erratic.
No. 744, from ATCC, 1939, No. 6051.
No. 745, from ATCC, 1939, No. 6598.
No. 968, from Porter, 1940; Bredemann; Gottheil. A greenish-yellow, fluorescent pigment produced on nutrient agar at 45° C. and a dark-pink, almost purple, growth on potato at 28°.
No. 969, from Porter, 1940; Stapp.
No. 971, from Porter, 1940; Bact. Dept., Iowa State Col.; Soule. A greenish-yellow fluorescent pigment produced on nutrient agar at 45° C.

No. 972, from Porter, 1940; Edwards; Marburg Univ. Germany; Cohn strain. Same as No. 968 above.
No. 975, from Porter, 1940; Edwards; Meyer; Cohn strain.
No. 978, from Porter, 1940; NCTC, No. 2586; Ford No. 1.
Discarded, from Blumberg, Columbia Univ., 1941.

Bacillus subtilis

From NIH, 1937, No. 60; Evans; Conn; Pribram. See *B. cereus* No. 538.
From Porter, 1940; Edwards; Conn. See *B. cereus* No. 973.
From Porter, 1940; Edwards; Conn; Chester strain. See *B. cereus* No. 974.
From Porter, 1940; Edwards; Novy. See *B. cereus* No. 976.

Bacillus subtilis, Michigan strain

From Soule, Mich. Univ., 1936; Ford. See *B. cereus* No. 303.
From NIH, 1937, No. 19. See *B. cereus* No. 537.
From Porter, 1940; Edwards; Soule. See *B. cereus* No. 970.

SYNONYMY

The following 26 named cultures that apparently conform to their original descriptions were identified by the writers as *Bacillus subtilis*.

Bacillus aterrimus

	B. subtilis No.
From Hall, Colo. Univ. Med. School, 1936, No. 798B; Ford	263
From NCTC, 1937, No. 2590; Ford No. 5A	330
From Cameron, NCA, 1937; Breed; NCTC No. 2590; Ford No. 5A	652
From Porter, 1940; NCTC No. 2590; Ford No. 5A	814

No. 263 failed to reduce nitrates in the routine test. After four serial transfers at weekly intervals to nitrate broth, nitrites were positive, becoming strong in the tenth serial transfer.

None of the latter three cultures (Nos. 330, 652, 814) produces the characteristic black pigment of *B. aterrimus* on potato or carbohydrate media. Since the writers have shown that the loss of pigmentation can be brought about (see section on Dissociation of *B. aterrimus*), it seems likely that these may be colorless dissociants of the original. This view is strengthened by the fact that Ford's No. 5B (see *B. subtilis* var. *aterrimus* No. 653) still produces the black pigment.

Bacillus aterrimus, from ATCC, 1939, No. 4509; Ford No. 5. See *B. cereus* No. 723.

Bacillus lactis niger

From Gorini, 1936, Milan, No. 1B ..... *B. subtilis* No. 255

For a discussion of this culture, see *B. niger* below.

Bacillus mesentericus

From Blumberg, Columbia Univ., 1941..... *B. subtilis* (discarded)

Bacillus mesentericus fuscus Flügge, Die Mikroorganismen, p. 321. 1886.

From Porter:	*B. subtilis* No.
1940; Bredemann	897
1940; Edwards; Marburg Univ.; Cohn	898

For a discussion of *B. mesentericus* and *B. mesentericus fuscus*, see *B. pumilus*.

Bacillus mesentericus hydrolyticus Hermann and Neuschul, Biochem. Ztschr. 281: 219–230. 1935.

From Porter, 1940; Hermann ..... *B. subtilis* No. 901

Isolated from carrots, this organism was reported as having a strong hydrolytic action on sugar and soluble starch and also softened and loosened the connective tissue of carrots, peas, cucumbers, radishes, and other vegetables. Culture No. 901 fails to show any special hydrolytic ability. Another strain received under the name of *B. vulgatus hydrolyticus* (see below) is identical, both being the intermediate rough-smooth stage of *B. subtilis*.

Bacillus mesentericus ruber (Globig) Kruse, *in* Flügge, Die Mikroorganismen, p. 199. 1886.
Potato bacillus, Globig, Ztschr. f. Hyg. u. Infektionskrank. 3: 322. 1888.

From Porter, 1940 ..... *B. subtilis* No. 900

This culture agrees with the rather meager descriptions given by Kruse, particularly in the production of a red color on potato. Instead of being rough, as are the majority of the strains of *B. subtilis*, it is smooth, colonies are thin, dendroid, spreading, and the vegetative rods are somewhat slender. In these respects it resembles *B. pumilus*, but in all other characters it is similar to *B. subtilis*. Migula (*77, p. 554*) changed the name to *B. globigii*. Two cultures with this name are in this collection and appear to be misnamed. One (NCTC, 1937, No. 2593; Ford No. 7; Kral) is *B. pumilus* No. 331, and the other (ATCC, 1936, No. 4516; Ford No. 7; Kral) is *B. circulans* No. 313.

Bacillus mesentericus ruber, from Porter, 1940; Torrey Res. Sta. See *B. cereus* No. 644.

Bacillus natto Sawamura, Tokyo, Nogyo Daigaku (Tokyo Agr. Col. Bul.) 7: 108. 1906. *See also* Tokyo Nogyo Daigaku (Tokyo Agr. Col. Jour.) 5: 789. 1913.

From Hanzawa, Imperial Univ., Tokyo:	*B. subtilis* No.
1930	351
1932	352

This organism is reputed to be the cause of the characteristic flavor of natto (fermented soybeans). Both cultures were identical with *B. subtilis*.

Bacillus niger

From Porter, 1940; Fabian; Gorini ..... *B. subtilis* No. 913

This strain and No. 255 above (*Bacillus lactis niger*) are probably duplicates. Neither of them produced a black pigment during this study. Since it has been shown above that loss of pigmentation occurs (see Dissociation of *B. niger*) it is probable that these cultures are authentic. (See *B. subtilis* var. *niger*.)

Bacillus panis Migula, Syst. der Bakt., p. 576. 1900.
B. mesentericus panis viscosus II Vogel, Ztschr. f. Hyg. u. Infektionskrank. 26: 404. 1897.

	B. subtilis No.
From NCTC, 1937, No. 2594; Ford No. 8	332
From Porter, 1940; NCTC No. 2594; Ford No. 8	926
From ATCC, 1936, No. 82; AMNH No. 796; Ford No. 5	315

There has been a great deal of confusion regarding the so-called "slimy-bread organism," caused by the inadequate early descriptions. Laubach (*60, p. 501*) identified an isolate as *B. panis*, which was encapsulated. After continued cultivation on artificial media it lost its capsules, although it remained rather slimy. Lehmann, Neumann, and Breed (*63, p. 615*), in discussing bacilli from slimy bread, state that

> many "species" of spore-forming bacteria are described which . . . are interrelated and also more or less closely related to *B. mesentericus* and *B. vulgatus*.

The duplicate cultures Nos. 332 and 926 are not encapsulated now, and no slime is formed in carbohydrate media. No. 315 is aberrant. The growth is extremely rough and slow at 28° C.; no growth at 40°. Its fermentation of carbohydrates is weak and no acetylmethylcarbinol is formed. A few inoculations into glucose broth and plating failed to develop a smooth strain. From the study of these cultures of *B. panis*, the writers conclude that it is a mucoid variant of *B. subtilis* and as such is not entitled to the rank of a variety, much less that of a species.

Bacillus panis, from ATCC, 1936, No. 4522; Ford No. 8. See *B. pumilus* No. 316.

Bacillus subtilis var. *viscosus* Chester, Del. Agr. Expt. Sta. Ann. Rpt. (1903): 84. 1904.

From Porter, 1940; NCTC No. 2587; Ford No. 2..... *B. subtilis* No. 979

Chester obtained a culture marked *B. subtilis* from Kral's Laboratory and another labeled *B. mesentericus-fuscus* from Bacteriology Laboratory, Johns Hopkins University, and found them identical with *B. subtilis*, except for the mucoid and viscid growth. He said, "This feature is so strongly developed as to make it worthy of special note." No. 979 forms a soft smooth growth but is not slimy. Its action on carbohydrates is somewhat slower than with rough strains of this species, but there is nothing about it worthy of special note.

Bacillus tenuis (Duclaux) Migula, Syst. der Bakt., p. 587. 1900.
Tyrothrix tenuis Duclaux, Le Lait [Paris], p. 218. 1894.

From Dubos, Rockefeller Inst. Med. Res.:	*B. subtilis* No.
1940; NCTC, No. 2118, var. A	776
1940; NCTC, No. 2119, var. B	777

Unfortunately, the writers were not able to obtain the original article. From the incomplete descriptions given by Duclaux and copied by Migula, it is difficult to recognize the organism. Chester (*14, p. 273*) said the growth was similar to *B. vulgatus*, and Lehmann, Neumann, and Breed (*63, p. 608*) placed it between *B. subtilis* and *B. vulgatus*. Both Nos. 776 and 777 are typical rough strains of *B. subtilis* and no doubt are authentic.

Bacillus vulgatus (Flügge) Trevisan, I Generi e Spec. d. Batteriacee, p. 19. 1889.
B. mesentericus vulgatus Flügge, Die Mikroorganismen, p. 322. 1896.

	B. subtilis No.
From Scales, USDA, 1922	237
From AMNH, 1923, No. 725; Ford	238
From NCTC, 1927, No. 1097; Ling	329
From Ark, Calif. Univ., 1941	787
From Porter, 1940	1003
From Porter, 1940; NCTC No. 2588; Ford No. 3	1004

A statement of the relationship of *B. vulgatus* to *B. subtilis* will be found above in the discussion of *B. subtilis*. Of the cultures listed above, three show the characteristic folded pigmented growth of *B. vulgatus*, whereas the other three show the finely wrinkled rough growth of *B. subtilis*. From the data on these cultures there seems to be no reason for making separate species.

Bacillus vulgatus hydrolyticus

From ATCC, 1939, No. 6984; Hermann ..... *B. subtilis* No. 747

This was introduced as *B. mesentericus hydrolyticus* (see above), a strongly hydrolytic variety of *B. mesentericus vulgatus*. Since No. 747 is identical with 901 (*B. mesentericus hydrolyticus*) and came from the same source, it is assumed that the name was changed to conform to the binomial *B. vulgatus*.

ASSIGNMENT OF OTHER NAMED CULTURES

The 33 cultures listed below do not conform to or are not recognizable from their original descriptions and have been identified by the writers as *Bacillus subtilis*.

Bacillus adhaerens Stühnk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 183. 1935.

From Porter, 1940; Bredemann ..... *B. subtilis* No. 802

Stühnk's characterization of this strain, isolated from the soil of a banana plantation, would seem to place it in the *B. circulans* group. The culture obtained is evidently not the original, because it is a typical *B. subtilis*. The name of this organism is a homonym because Laubach (*60, p. 503*) described a species under this same name, which he said slightly resembled *B. mycoides*. (See *B. adhaerens* in the assigned cultures under *B. pumilus*.)

Bacillus butyricus Hueppe, Mitt. aus dem K. Gesundheitsamte 2: 353. 1884.

From Porter, 1940; NCTC No. 1650; Kral ..... *B. subtilis* No. 820

From investigations on a stock culture maintained for a long time, Lehmann, Neumann, and Breed (*63, p. 610*) state that this organism stands between *B. megatherium* and *B. mesentericus*, and in some respects is like *B. vulgatus*. No. 820 appears to be similar to their culture, showing a fairly smooth rather than the typical rough growth. It is also slightly slower and weaker on various media.

Bacillus calidolactis Hussong and Hammer, Jour. Bact. 15: 186. 1928.

From Porter, 1940; NCTC No. 2690 ..... *B. subtilis* No. 821

This is not the original strain, which was a thermophile with an optimum temperature of 55° to 65° C. and no growth at 37°.

Bacillus danicus

From Porter, 1937; Glathe .....*B. subtilis* No. 601

This culture is definitely misnamed. It does not agree with the description or with authentic cultures. (See synonomy of *B. megatherium*, Nos. 245 and 246.)

Bacillus filiformis (Duclaux) Tils, Ztschr. f. Hyg. u. Infectionskrank. 9: 294. 1890.
Tyrothrix filiformis Duclaux, Le Lait, p. 249. 1887.

From Dubos, 1940; NCTC No. 2120 .....*B. subtilis* No. 778

It is not possible to recognize Duclaux' culture from the description or from his illustrations, so no conclusions can be drawn as to the authenticity of No. 778.

Bacillus geniculatus De Bary, Beitrag zur Kenntnis der niederen Organismen im Mageninhalt, Inaug. Diss., Strassburg and Leipzig. 1885.

From Dubos, 1940; NCTC No. 2123 .....*B. subtilis* No. 772

Migula (*77, p. 525*) noted no difference between this organism and *B. subtilis* except in the smaller diameter of the rods (0.5μ to 0.6μ). Lehmann, Neumann, and Breed (*63, p. 608*) place it as a synonym of *B. parvus*. They also list (*63, p. 616*) *B. geniculatus* Duclaux (*Tyrothrix geniculata* Duclaux) (*34, p. 231*) as a relative of *B. panis*, the "slimy-bread" bacillus. In either event, although not recognizable from the early accounts, it is probable that No. 772 is very similar to if not identical with the original strains.

Bacillus graveolens

From ATCC, 1939, No. 7003; Orcutt .....*B. subtilis* No. 730

Authentic cultures are listed under the synonymy of *B. megatherium*.

Bacillus luteus

From Porter, 1937; Bredemann; Garbowski .....*B. subtilis* No. 619

Evidently misnamed; see the list of other named species assigned to *B. cereus*, p. 47.

Bacillus megatherium

B. subtilis No.
From ATCC, 1939, No. 943; Levine No. 2062 737
From Blumberg, Columbia Univ., 1941 .....discarded

Both cultures are mislabeled; they are probably duplicates. See *B. megatherium*.

Bacillus (Tyrothrix) minimus Duclaux, Le Lait, p. 213. 1887.

From Dubos, 1940; NCTC No. 2122; Rosenthal; Duclaux..*B. subtilis* No. 773

This name seems not to have been used by bacteriologists, and no discussion of the possible relation of this organism to others has been found in the literature. This is reputed to be the original strain. If so, it was identical with *B. subtilis*.

Bacillus musculi

From Porter, 1940 Claussen; Stapp ..... *B. subtilis* No. 909

For authentic cultures see *B. musculi* in the list of synonyms of *B. megatherium*.

Bacillus nitroxus Beijerinck, Centbl. f. Bakt. [etc.] Abt. 2, 25: 49. 1909.

From Porter, 1940; Kluyver .....*B. subtilis* No. 918

Beijerinck found rod, coccus, yeast forms, and clostridia in his culture, depending upon the medium used. No. 918 is quite different from his description.

Bacillus orae Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 464. 1933.

From Porter, 1940; Bredemann .....*B. subtilis* No. 919

The original account would indicate that this organism belongs to the *B. circulans* group; it definitely does not apply to *B. subtilis*.

Bacillus petasites

	B. subtilis No.
From NCTC, 1937, No. 2606; Ford No. 20	338
From Porter, 1940; Bredemann	930
From Porter, 1940; Claussen	933

These cultures are misnamed; see *B. petasites* in the synonymy of *B. megatherium*.

Bacillus pumilus

	B. subtilis No.
From ATCC, 1939, No. 4344	741
From Porter, 1940; Claussen	941

These cultures reduce nitrates to nitrites and hydrolyze starch, which is not done by *B. pumilus*. The growth is rough. See description of *B. pumilus*.

Bacillus robur

From Porter, 1937; Bredemann .....*B. subtilis* No. 631

Misnamed; see *B. robur* in the synonymy of *B. cereus*.

Bacillus rugulosus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 181. 1935.

From Porter, 1940; Bredemann .....*B. subtilis* No. 949

Stührk reported that nitrites were not formed from nitrates, although the growth on potato resembled *B. subtilis*. Other characters given by him do not help in determining the authenticity of No. 949.

Bacillus ruminatus

From Porter, 1937; Bredemann .....*B. subtilis* No. 634

For authentic cultures, see the synonymy of *B. megatherium*.

Bacillus scaber (Duclaux) Migula, Syst. der Bakt., p. 586. 1900.
Tyrothrix scaber Duclaux, Ann. Inst. Nat. Agron. 4: 94. 1882.

From Dubos:	*B. subtilis* No.
1940; NCTC No. 2116, var. A.	774
1940; NCTC No. 2117, var. B.	775

The writers have not been able to obtain the original description by Duclaux. In a later work (*34, p. 236*) he stated that the granular vegetative rods were 1.1μ to 1.2μ in diameter. His illustrations show that it resembled *B. cereus*, which, combined with the size of the rods, would lead one to believe that Nos. 774 and 775 are not authentic.

Bacillus serrulatus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 193. 1935.

From Porter, 1940; Bredemann .....*B. subtilis* No. 955

This culture bears no resemblance to Stührk's account of this organism, which apparently belonged to the *B. circulans* group.

Bacillus silvaticus

From Porter, 1937; Bredemann .....*B. subtilis* No. 636

Authentic cultures are listed in the synonymy of *B. megatherium*.

Bacillus simplex

From Porter, 1940; Bredemann .....*B. subtilis* No. 959

See *B. megatherium—B. cereus* intermediates.

Bacillus sphaericus

From Porter, 1940; Bredemann .....*B. subtilis* No. 963

Misnamed; no resemblance to *B. sphaericus* of group 3.

Bacillus tardivus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 177. 1935.

From Porter, 1940; Bredemann .....*B. subtilis* No. 981

The original apparently belonged to the round-spored group (*B. sphaericus* complex) and was quite different from the present culture.

Bacillus teres

From Porter, 1940; Claussen ..... *B. subtilis* No. 987

Each of the three cultures bearing this name is a different species, and no definite information is available to determine whether any of them are authentic. (See group B of the *B. megatherium–B. cereus* intermediates and the list of cultures assigned to *B. pumilus*.)

Bacillus terminalis

From Porter, 1940; NCTC No. 2612; Ford No. 28..... *B. subtilis* No. 983

Ford's No. 28 obtained from another source is *B. circulans* No. 746 (see group 2) and seems to represent the original.

Bacillus tumescens

From Porter, 1940; Bredemann ..... *B. subtilis* No. 989

This culture is misnamed; see synonymy of *B. megatherium*.

Bacillus watzmannii Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 462. 1935.

From Porter, 1937; Bredemann ..... *B. subtilis* No. 642

The spores were said to have a variety of shapes and sizes and the sporangia to be club- or spindle-shaped. In general, it seems to resemble the *B. circulans* complex. Culture 642 is, therefore, not the original.

IDENTIFICATION OF UNNAMED CULTURES

The following unnamed cultures were studied during the progress of the work and were found to be typical for *Bacillus subtilis*.

Unnamed cultures	*B. subtilis* No.
From NIH, 1937, Nos. 21, 30–32, 38, 40, 57, 58, 37, 54, and 115	551–559, 579, 581
From Reid, Pa. State Col., 1937, Nos. C1, B2, E11, from fermenting tobacco	698–700
From Cordon, N. J. Agr. Expt. Sta., 1938	702
From Presley, Sacaton, Ariz., 1938	703
From Curran, USDA, 1938	705
From Cohen, Harvard Univ., 1938	714
From Myers, Natl. Dairy Labs., Baltimore, Nos. RBKr. 5, RBKr. 5–Y, RB. 7008, and RB. 13A, from ropey bread	762–764, 766
From Blumberg, Columbia Univ., 1941, Nos. 1, 2, 6, and 7	789–792

No. 714 failed to reduce nitrates to nitrites in the routine test. After 10 serial transfers made at weekly intervals, nitrites were very strong. (Cf. *B. aterrimus* No. 263 in the synonymy above.)

Six other strains submitted by Blumberg (Nos. 3–5, 8–10) were identified as *B. subtilis* but were not placed in the collection. Schweiger, Ark. Univ., 1942, supplied 11 strains, which he had isolated from animal lesions, but, since they were typical for *B. subtilis*, they were not placed in the collection either.

Three isolations made by the writers from soil, flour, and slimy cake were identified as *B. subtilis* (Nos. 242, 712, and 713, respectively).

Bacillus subtilis var. *aterrimus*

Bacillus subtilis var. *aterrimus*, new combination.

Potato bacillus Biel, Centbl. f. Bakt. [etc.] Abt. 2, 2: [137]–140. 1896.

B. mesentericus niger Lunt, Centbl. f. Bakt. [etc.] Abt. 2, 2: 572–573. 1896.

B. aterrimus Lehmann and Neumann, Atlas und Grundriss der Bakt., p. 303. 1896.

In 1896 Lehmann and Neumann gave the name *Bacillus aterrimus* to the potato bacillus of Biel and to *B. mesentericus niger* Lunt, both of which had been reported that same year. The identity of *B. aterrimus* was generally confused with that of *B. lactis niger* Gorini (*B. niger*), until the investigation of Clark and Smith (*20*) in 1939. They found that the two species could be separated by their cultural requirements for pigment production, as *B. aterrimus* blackens only carbohydrate media whereas *B. niger* produces the black pigment only in the presence of tyrosine.

In the section dealing with Studies on Dissociation it was shown that both of these organisms may lose their ability to form the black pigment. These colorless dissociants are identical with *B. subtilis* in morphology, physiology, and in the character of the growth. They remain colorless once they are stabilized.

The description of *B. subtilis* will serve for var. *aterrimus* with the addition that a blue-black to black pigment is produced in media containing a carbohydrate that is readily utilized by the organism.

TYPE CULTURE STUDIED

The following type culture was studied and found to conform to the characterization of *Bacillus aterrimus*.

Bacillus aterrimus

No. 653, from Cameron, NCA, 1937; Breed; NCTC No. 2591; Ford No. 5B.

This was the only authentic named culture obtained of *B. aterrimus* that still produced the black pigment. Ford's culture No. 5A fails to show any black pigment and has been listed under *B. subtilis* (Nos. 330, 652, and 814), with the notation that it is probably a colorless dissociant of the parent strain.

Bacillus aterrimus

From Hall, Colo. Univ. Med. School, 1936, No. 798B; Ford. See *B. subtilis* No. 263.
From ATCC, 1936, No. 945; Levine No. 2081. See *B. pumilus* No. 266.
From Schooley, Bucknell Univ., 1936. See *B. megatherium* No. 267.
From ATCC, 1939, No. 944; Levine No. 2025. See *B. cereus* No. 722.
From ATCC, 1939, No. 4509; Ford No. 5. See *B. cereus* No. 723.

SYNONYMY

Named cultures that apparently correspond to their original descriptions were identified as *Bacillus subtilis* var. *aterrimus* as follows.

Bacillus nigrificans Fabian and Nienhuis, Mich. Agr. Expt. Sta. Tech. Bul. 140: 23–27. 1934.

	B. subtilis var. *aterrimus* No.
From Porter, 1937; Fabian	624
From Cameron, NCA, 1937; Fabian	659
From ATCC, 1939, No. 4925; Fabian	740

Fabian and Nienhuis reported that *B. nigrificans* was similar to *B. niger* and *B. mesentericus* in many respects and that its distinguishing character was the formation of a water-soluble black pigment in glucose brine. The above three cultures, all originally from Fabian, blacken carbohydrate media and are clearly authentic.

ASSIGNMENT OF A NAMED CULTURE

The following strain is not recognizable from its description and has been identified as *Bacillus subtilis* var. *aterrimus*.

Bacillus tyrosinogenes Rusconi, *in* Carbone and others, Biol. d. Inst. Sieroterap., Milan 2: 29–46. 1921.

From Hall, Colo. Univ. Med. School, 1937; Institute Sieroterap., Milan ..... *B. subtilis* var. *aterrimus* No. 353

This organism is not to be confused with the anaerobe, *Clostridium tyrosinogenes* (Hall) Bergey and others (*6*). In their study of the serology of *B. aterrimus*, *B. mesentericus fuscus*, and *B. carotarum*, Carbone and others included *B. tyrosinogenes* and stated that it had been isolated by Rusconi in 1912. The serological results indicated a close relation among all four species but provided no clue as to the identity of *B. tyrosinogenes*. This is the only reference to Rusconi's organism found by the writers.

IDENTIFICATION OF UNNAMED CULTURES

The following unnamed cultures were identified as *Bacillus subtilis* var. *aterrimus*.

Unnamed cultures	*B. subtilis* var. *aterrimus* No.
From Thom, USDA, 1936, from cornstarch in 1912	230
From Hall, Colo. Univ. Med. School, 1936, Nos. 581, 620, 621A, and 622	259–262

Three strains of *B. subtilis* var. *aterrimus* (Nos. 274–276) were isolated by the writers from soil and air and another strain (No. 748) from decomposed wheat grain.

Bacillus subtilis var. *niger*

Bacillus subtilis var. *niger*, new combination.

B. lactis niger Gorini, R. Soc. Ital. d'Ig., Gior. 16: 9. 1894; Centbl. f. Bakt. [etc.] Originale (1) 20: 94. 1896.

B. niger Migula, Syst. der Bakt., p. 363. 1900.

Gorini first named his organism *Bacillus nero*, then changed it to *B. lactis niger* (*44*). He described it as a large rod, 1.3μ by 5μ to 7μ, that blackened potato and agar. Sometime later, he divided the culture into the A and B strains. Neither of these now produce the black pigment. Strain No. 1A is *B. cereus* (Nos. 256 and 654); it ferments lactose and agrees with Gorini's original account except for lack of pigment. Strain No. 1B is considerably smaller (0.7μ by 2μ to 4μ) and is a typical *B. subtilis* (Nos. 255 and 913). Of course, this might be a colorless dissociant of the original pigmented strain that had become mixed with *B. cereus*. If so, the accident must have happened early, because in 1895 Lehmann and Neumann (*62, p. 617*) failed to get pigment formation in Gorini's culture obtained through Kral's laboratory. Chester (*15, p. 306*) listed Biel's black potato bacillus (*B. aterrimus*) as *B. niger*, thus adding to the confusion. Lawrence and Ford (*61*) obtained from Kral a culture of *B. niger* that blackened nutrient agar. It is interesting that their culture obtained through Hall (No. 264 below) and also Kral's culture obtained through the AMNH (No. 220 below) still form the black pigment.

The characterization of *B. subtilis* will serve for *B. subtilis* var. *niger* by adding the words "substrate blackened" to the description of the growth on media that contain tyrosine.

TYPE CULTURES STUDIED

The following seven authentic cultures were studied and identified as *Bacillus subtilis* var. *niger*.

Bacillus niger

No. 220, from AMNH, 1923, No. 733; Kral.
No. 264, from Hall, Colo. Univ. Med. School, 1936, No. 799; Ford; Kral.
No. 650, from Cameron, NCA, 1937; Breed; NCTC No. 2736; Gorini.
No. 651, from Cameron, NCA, 1937; Breed; NCTC No. 2592; Ford No. 6.

Bacillus lactis niger

No. 254, from Gorini, Milan, 1936, No. 2.
No. 655, from Cameron, NCA, 1937; Breed; Gorini No. 2.

Bacillus subtilis niger

No. 704, from Rakieten, Hoagland Lab., Brooklyn, N. Y., 1937, No. C3.

Bacillus niger

From Cameron, 1937; Breed; Levine No. E-17. See *B. megatherium–B. cereus* intermediate No. 656.
From Cameron, 1937; Breed; Robertson Nos. 1 and 2. See *B. pumilus* Nos. 657 and 658.
From Porter, 1940; Fabian; Gorini. See *B. subtilis* No. 913.

Bacillus lactis niger

From Gorini, 1936, No. 1B. See *B. subtilis* No. 255.
From Gorini, 1936, No. 1A. See *B. cereus* No. 256.
From Cameron, 1937; Breed; Gorini 1A. See *B. cereus* No. 654.

IDENTIFICATION OF UNNAMED CULTURES

One unnamed culture from Hall, Colo. Univ. Med. School, 1936, was identified as *Bacillus subtilis* var. *niger* No. 265. Twenty-one isolations made by the writers from soil and air were also identified as *B. subtilis* var. *niger*, Nos. 211–219, 221–229, 253, 356, and 357.

Bacillus subtilis–Bacillus pumilus intermediates

Although the rods of *Bacillus subtilis* are usually larger than those of *B. pumilus*, the two species cannot be differentiated microscopically because of the variability in size found in many strains of both species. Normally, *B. subtilis* is rough and *B. pumilus* is smooth. The converse frequently occurs, however, making it necessary to base the separation on biochemical reactions. Of these, the hydrolysis of starch and the reduction of nitrates to nitrites are the outstanding differences. *B. subtilis* attacks starch and forms nitrites from nitrates; *B. pumilus* does neither.

It should be expected that intermediates between two species so closely related would be found if sufficient cultures were studied. Two isolates in the present collection (Nos. 754, 755) are placed here, because they hydrolyze starch but do not reduce nitrates to nitrites. They were transferred serially in nitrate broth every week for 20 times and still remained negative, a procedure that induced nitrate reduction with *B. subtilis* No. 263 (*B. aterrimus* in the synonymy) and with *B. subtilis* No. 714 (unnamed culture).

In addition to the above, Nos. 754 and 755 are susceptible to the phage for *B. pumilus*. They are, therefore, closely related to the latter and perhaps might be considered biotypes rather than intermediates. They were both isolated from soil by the writers.

Bacillus pumilus

Bacillus pumilus Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 681. 1901.

Gottheil stated that the growth of *Bacillus pumilus* on agar was thin, shiny and vitreous, which turned yellowish upon aging. Starch was not hydrolyzed. Chester (*15, p. 87*) and Lawrence and Ford (*61, p. 300*) regarded it as identical with their cultures of *B. mesentericus*. Their conclusions are substantiated by the present work. Unfortunately, the strain of *B. mesentericus* that they had is not the same as the one accepted in Europe under that name. In

1939, T. Gibson,[14] University of Edinburgh, stated that the European strain of *B. mesentericus* ferments starch and reduces nitrates to nitrites. His observations were to have been published but have not appeared so far as the writers are aware.

The original description of *B. mesentericus fuscus* by Flügge (*35, p. 321*) is quite indefinite but apparently applied to a rather smooth organism. Trevisan (*108, p. 19*) changed the name to *B. mesentericus* but added nothing. Later, Flügge (*36, p. 294*) said the growth was luxuriant and had a dull waxlike sheen, becoming dry and wrinkled. Whether Flügge was describing two different organisms (in the first instance a culture similar to *B. pumilus*, and in the second a culture resembling *B. subtilis*) cannot be stated and the writers would hesitate to give that as the reason for the confusion between the American and European strains of *B. mesentericus*. Since such a situation exists and since the European strain is in synonymy with *B. subtilis*, it is recommended that the name *B. mesentericus* be considered nomen dubium and that the American strain of *B. mesentericus* hereafter be known as *B. pumilus*.

The following description of *B. pumilus* is based upon the study of 4 type cultures, 4 strains that bore names in synonymy, 42 cultures that had other names, and 20 that were received unnamed, a total of 70 strains.

CHARACTERS

**Vegetative rods.*—0.6μ to 0.7μ by 2.0μ to 3.0μ; cells stain evenly and usually occur singly; ends rounded; no capsules and no shadow-forms; motile; Gram-positive. *Variations:* 0.5μ to 1.0μ by 1.0μ to 5.0μ; chains of cells and long filaments; shadow-forms; few strains encapsulated; or Gram-variable.

On glucose-nutrient agar the cells contain a few small fat globules. There is a tendency toward larger cells, more filaments, and Gram-negative forms.

**Sporangia.*—Not definitely bulged.

**Spores.*—0.5μ by 1.0μ; oval to cylindrical; central to paracentral; naked, freely formed. *Variations:* 0.6μ to 0.8μ by 0.8μ to 1.5μ; few appear barrel-shaped; or sporulation slow, 5 to 14 days; two strains sporulate only on soybean agar.

Colonies.—Smooth; thin; flat; spreading; dendroid; translucent. *Variations:* Small, nonspreading; dense; or pinpoints.

Nutrient agar slants.—Growth smooth; thin; glistening; spreading; nonadherent. *Variations:* Rough; dull; tough; wrinkled; or yellowish.

**Glucose-nutrient agar slants.*—Usually similar to nutrient agar, although growth may be rougher and heavier in some cases.

**Glucose-nitrate agar slants.*—Growth scant; good growth by nine strains.

**Tyrosine agar slants.*—Same as nutrient agar.

Potato.—Growth smooth; thin; soft; spreading; moist to slimy; yellowish turning to brownish; water sometimes has pellicle. *Variations:* Growth rough; dry; wrinkled; pink; or greenish-yellow pigment in water.

Nutrient broth.—Turbidity uniform, with or without a ring or

[14] See footnote 13, p. 53.

soft pellicle. *Variations:* Growth flocculent with rough pellicle or broth clear with dry rough pellicle.

**Voges-Proskauer reaction.*—Positive; one strain negative.

Utilization of citrate.—Positive.

**Fermentation tests.*—Acid formed from arabinose, xylose, glucose, fructose, galactose, mannose, sucrose, salicin, glycerol, and mannitol; usually positive on maltose and raffinose; variable on dextrin; usually negative on rhamnose, lactose, and inulin.

**Starch hydrolysis.*—Negative (two starch positive variants listed as *B. subtilis–B. pumilus* intermediates).

**Nitrites from nitrates.*—Negative.

Maximum temperature for growth.—50° C. *Variations:* Some failed to grow above 40° C.; a few will grow at 52°.

Gelatin hydrolysis.—Positive; slow liquefaction in tubes of gelatin in 5 days; more rapid change by the Frazier plate method.

Casein hydrolysis.—Positive.

TYPE CULTURES STUDIED

The following authentic cultures were received and identified as *Bacillus pumilus.*

Bacillus pumilus

No. 272, from Löhnis, Leipzig, 1914; Kral.
No. 630, from Porter, 1937; Bredemann.
No. 939, from Porter, 1940; Bredemann, var. A.
No. 940, from Porter, 1940; Stapp.

Bacillus pumilus

From ATCC, 1939, No. 4344. See *B. subtilis* No. 741.
From Porter, 1940; Claussen. See *B. subtilis* No. 941.
From Porter, 1940; Claussen. See *B. cereus* No. 942.

SYNONYMY

The following named strains that conform to their descriptions were identified by the writers as *Bacillus pumilus.*

Bacillus mesentericus (Flügge) Trevisan, I Generi e Spec. d. Batteriacee, p. 19. 1889.

B. mesentericus fuscus Flügge, Die Mikroorganismen, p. 321. 1886.

	B. pumilus No.
From AMNH, 1923, No. 726; Ford	236
From Soule, Mich. Univ., 1936	307
From ATCC, 1939, No. 945; Levine No. 2081	738
From Porter, 1940; Edwards; ATCC	896

ASSIGNMENT OF OTHER NAMED CULTURES

The 42 cultures listed below do not conform to their original description or are not recognizable because of the inadequacy of their characterizations. They were studied in detail and identified as *Bacillus pumilus.*

Bacillus adhaerens Laubach, Jour. Bact. 1: 503. 1916.

	B. pumilus No.
From ATCC, 1936, No. 1; AMNH; Ford	309
From NCTC, 1937; No. 2604; Ford 18	337

Laubach stated that young colonies somewhat resembled *B. mycoides,* growth on agar slants extended into the medium, and the diameter of the spores was greater than that of the rods. These characters certainly do not apply to the cultures at hand. Lacking cultures that correspond with the original account the writers admit that it is impossible to allocate Laubach's *B. adhaerens.* (See also *B. adhaerens* in the assigned cultures under *B. subtilis.*)

Bacillus agri Laubach and Rice, Jour. Bact. 1: 516. 1916.

From NCTC, 1937, No. 2598; Ford No. 12 ..... *B. pumilus* No. 336

This culture does not agree with two other cultures under this name obtained from ATCC (Nos. 2 and 4507), both of which are *B. cereus* (Nos. 342 and 310, respectively). The original account is incomplete and does not apply in all respects to either the ATCC or the NCTC strains.

Bacillus agrophilus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 189. 1935.

From Porter, 1940; Bredemann ..... *B. pumilus* No. 807

This culture is evidently misnamed. The original account, although incomplete, would indicate that it belonged to the *B. brevis* group.

Bacillus aterrimus

From ATCC, 1936, No. 945; Levine No. 2081 ..... *B. pumilus* No. 266

For authentic cultures, see *B. subtilis* var. *aterrimus.*

Bacillus brevis

From ATCC:	*B. pumilus* No.
1939, No. 18; AMNH; Ford	724
1939, No. 4510; Ford No. 27	725

Both are misnamed; see cultures under *B. brevis.*

Bacillus carotarum

From Porter, 1940; Stapp ..... *B. pumilus* No. 827

Culture is misnamed; see *B. carotarum* in the synonymy of *B. megatherium.*

Bacillus cohaerens

From NCTC, 1937, No. 2596; Ford No. 10A; Kral ..... *B. pumilus* No. 334

This culture is misnamed; see *B. megatherium–B. cereus* intermediates, group A.

Bacillus dendroides Thornton, Ann. Appl. Biol. 9: 247. 1922.

From Porter, 1940; NCTC No. 927; Thornton ..... *B. pumilus* No. 844

Thornton believed that his isolation was identical with *B. dendroides* described in 1909 by Holzmüller (*50, p. 331*). There is considerable doubt of this, however, because Holzmüller was studying *B. mycoides* and in addition to designating four varieties by Greek letters he named four new species, of which *B. dendroides* was one. He gave the diameter of the vegetative rods as 0.75μ to 1.21μ, with ends of the rods square. On the other hand, Thornton gave the diameter of the rods of his culture as 0.5μ and the diameter of the spores as 0.75μ, which would make a bulging sporangium in contrast to the nonbulging sporangium of Holzmüller's culture. Thornton also stated that starch was hydrolyzed and nitrates were reduced to nitrites. No. 844 is apparently not Thornton's original culture, because the sporangium is not bulged, starch is not hydrolyzed, and nitrates are not reduced to nitrites.

Bacillus fastidiosus Dooren de Jong, Zentbl. f. Bakt. [etc.] Abt. 2, 79: 350. 1929.

From Porter, 1937; Dooren de Jong No. 1071 ..... *B. pumilus* No. 643

This culture is obviously mislabeled, since it bears no resemblance to the original account. It was said to be a large (2.0μ in diameter) Gram-negative rod that utilized only the carbon from uric acid and would not grow on peptone.

Bacillus fusiformis

	B. pumilus No.
From AMNH, 1920, No. 732	247
From Porter, 1940; Bredemann	864
From Porter, 1940; Stapp	865

In 1937 No. 247 was separated from *B. fusiformis* No. 350 with which it was mixed. For authentic cultures, see *B. sphaericus* var. *fusiformis.*

Bacillus globigii Migula, Syst. der Bakt., p. 554. 1900.

Red potato bacillus, Globig, Ztschr. f. Hyg. u. Infektionskrank. 3: 322. 1888.

B. mesentericus ruber Kruse in Flügge, Die Mikroorganismen, p. 199. 1896.

From NCTC, 1937, No. 2593; Ford No. 7; Kral ..... *B. pumilus* No. 331

This culture does not produce a red color nor would it be mistaken for a "potato bacillus." For a probable authentic culture, see *B. mesentericus* var. *ruber* in the synonymy of *B. subtilis.*

Bacillus globigii, from ATCC, 1936, No. 4516; Ford No. 7, Kral. See *B. circulans* No. 313.

Bacillus lacticola

From Porter, 1940; Claussen .....*B. pumilus* No. 878

This culture is misnamed; see *B. lacticola* in the synonymy of *B. cereus.*

Bacillus liodermis Flügge, Die Mikroorganismen, p. 323. 1886; Ztschr. f. Hyg. u. Infektionskrank. 17: 296. 1894.

From ATCC, 1939, No. 70; AMNH; Winslow .....*B. pumilus* No. 734

Flügge's account is too meager for the identification of his culture. Chester (*14, p. 272*) and Lehmann, Neumann, and Breed (*63, p. 619*) consider it closely related to *B. vulgatus,* i. e., *B. subtilis* of the present work.

Bacillus luteus

From Porter, 1940; Stapp .....*B. pumilus* No. 885

See the assignment of other named cultures under *B. cereus* (No. 886).

Bacillus luteus, from Porter, 1937; Bredemann. See *B. subtilis* No. 619.

Bacillus malabarensis

From Porter:	*B. pumilus* No.
1940; Bredemann	620
1940; Claussen	891

For authentic culture, see *B. malabarensis* in the synonymy of *B. megatherium.*

Bacillus megatherium

From ATCC:	*B. pumilus* No.
1939, No. 71; AMNH No. 734A; Ford	735
1939, No. 72; Ford; Kral	736

These two cultures seem to be the victims of some error. In 1923 the writers obtained a typical culture of *B. megatherium* from the AMNH No. 734, and it is in the present collection as No. 234. In the 1928 catalog of the ATCC, *B. megatherium* Nos. 71 and 72 are listed as coming from AMNH Nos. 734A and 734B, respectively, but in the 1938 catalog No. 72 is said to come from Kral through Ford. No. 736 is the only strain of *B. pumilus* in the collection that does not produce acetylmethylcarbinol.

Bacillus mesentericus flavus Laubach, Jour. Bact. 1: 497. 1916.

	B. pumilus No.
From NCTC, 1937, No. 2595; Ford No. 9	333
From ATCC, 1939, No. 4520; Ford No. 9	739

This variety was named by Laubach for the yellow pigment it produced. The two cultures above are no longer pigmented. It is possible, of course, that they may be colorless dissociants of the original.

Bacillus niger

From Cameron, 1937; Breed; Robertson Nos. 1 and 2
.....*B. pumilus* Nos. 657 and 658

Authentic cultures are listed under *B. subtilis* var. *niger.*

Bacillus nigrificans

From Porter, 1937; Fabian .....*B. pumilus* No. 647

The above strain of *B. pumilus* occurred as a contaminant of the original culture, which is *B. subtilis* var. *aterrimus* No. 624.

Bacillus panis

	B. pumilus No.
From Blumberg, Columbia Univ., 1941	Discarded
From ATCC, 1936, No. 4522; Ford No. 8	316

A discussion of *B. panis* will be found under that name in the synonymy of *B. subtilis.*

Bacillus parvus Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 344. 1904.

From Porter:	*B. pumilus* No.
1937; Bredemann	629
1940; Claussen	928

Neide found that his culture hydrolyzed starch, a character absent in the cultures above. There is some doubt, therefore, about their origins, especially since Neide thought that *Tyrothrix tenuis* was a synonym. The latter is now regarded as synonymous with *B. subtilis.*

Bacillus pseudotetanicus

From Porter, 1940; NCTC No. 2609; Ford No. 25 .....*B. pumilus* No. 937

This organism is misnamed and is discussed under *B. pseudotetani* in the list of assigned cultures following *B. sphaericus.*

Bacillus ruminatus

From ATCC, 1936, No. 98; AMNH No. 793; Ford .....*B. pumilus* No. 345

Authentic cultures will be found in the synonymy of *B. megatherium.*

Bacillus sublustris Schieblich, Centbl. f. Bakt. [etc.] Abt. 2, 58: 206. 1923.

From Porter, 1940; Schieblich .....*B. pumilus* No. 980

According to Schieblich the vegetative cells were 0.7μ to 1.0μ in diameter, had rounded ends, and usually swelled before the round terminal spores formed. The growth was restricted, smooth, and shiny on agar. No acid or gas was formed on glucose. All these characters point to the *B. sphaericus* group; certainly not to *B. pumilus.*

Bacillus teres

From Porter, 1937; Bredemann .....*B. pumilus* No. 637

Bacillus teres is discussed in group B of the *B. megatherium–B. cereus* intermediates.

Bacillus terminalis var. *thermophilus* Prickett, N. Y. State Agr. Expt. Sta. Tech. Bul. 147: 44. 1928.

From Porter, 1940; NCTC No. 2688; Conn .....*B. pumilus* No. 984

Prickett stated that this variety had all the characters of *B. terminalis* Migula (*77, p. 578*) with the exceptions of a brown coloration on agar and a much higher optimum temperature (55° to 65° C.). Lawrence and Ford (*61, p. 314*) added to Migula's account, and it would appear that they had a culture of *B. brevis.* In any case, the culture received by the writers was mislabeled.

Bacillus terrestris Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 461. 1933.

From Porter, 1937; Bredemann .....*B. pumilus* No. 638

This culture is evidently not the original, because Werner stated that the spores were round and the shape of the sporangium was drumstick, neither of which applies here.

Bacillus thermophilus Rabinowitsch, Ztschr. f. Hyg. u. Infektionskrank. 20: 154. 1895.

From Porter, 1940; NCTC No. 2812; Pribram; Nègre ....*B. pumilus* No. 982

Since Nègre (*80*) found the maximum temperature for growth to be 70° C., No. 982, which fails to grow at 52°, is evidently not the original.

Bacillus tritus Batchelor, Jour. Bact. 4: 29. 1919.

From Henry, Wash. [State] Univ., 1937, No. 193 .....*B. pumilus* No. 667

Batchelor gave the diameter of the rods as 0.75μ, whereas the present culture is 0.5μ to 0.6μ. She also said that gelatin was not liquefied and no acid was formed from glucose and sucrose, all of which are positive with No. 667. Other

points of her rather brief characterization are not at variance with the present culture, which may or may not be authentic.

Bacillus truffauti Truffaut and Bezssonoff, Acad. des Sci. Colon. Paris, Compt. Rend. 175: 544–546. 1922.

	B. pumilus No.
From McCoy, Wis. Univ., 1937; Truffaut	355
From Porter, 1940; NCTC No. 1490	997

These two cultures are not original, because they do not reduce nitrates or hydrolyze starch; nor do they form a dry pellicle on broth as stated by Truffaut and Bezssonoff. Their organism also fixed atmospheric nitrogen, which further complicates its identity. Aside from the fixation of nitrogen, the size of the rods and other characters given by them would indicate a strain of *B. subtilis.*

Bacillus tumescens

From Porter, 1940; NCTC No. 2607; Ford No. 5A .....*B. pumilus* No. 990

See *B. tumescens* in the synonymy of *B. megatherium.*

IDENTIFICATION OF UNNAMED CULTURES

The following 20 unnamed cultures were submitted to the writers during the course of the work and have been identified after a complete study as strains of *Bacillus pumilus.*

Unnamed cultures

	B. pumilus No.
From NIH, 1937, Nos. 5, 43, 44, 56, 197, 201, 212	576–578, 580, 582–584
From Burkey, USDA, 1938, Nos. 1, 2, 4, 5, 12, 14	706–711
From Ark, Calif. Univ., 1941	788
From Weintraub, Smithsonian Inst., 1943	1034, 1035
From Branch, McGill Univ., 1940, Nos. 2, 18, 595, 801	Discarded

Bacillus coagulans

Bacillus coagulans Hammer, Iowa Agr. Expt. Sta. Res. Bul. 19: 129. 1915; Sarles and Hammer, Jour. Bact. 23: 301–314. 1932.

Bacillus coagulans was introduced by Hammer in 1915 as the cause of an outbreak of coagulation in evaporated milk. In 1932, Sarles and Hammer made a more detailed study of it, confining their observations on its occurrence to its presence in evaporated milk. The strains studied by the writers seem to be intermediate between the mesophilic and the thermophilic sporeformers. They prefer a temperature of 45° to 50° C., some of them growing at 60°, and growing poorly, if at all, at 25°. An unusual quantity of acid is formed from carbohydrates; the nonvolatile acid being *d* lactic acid and the volatile apparently acetic and propionic acids (*92*). In morphology they resemble *B. subtilis* to some extent, although the rods are usually longer and the spores usually terminal.

The following description of *B. coagulans* is based upon five type cultures—two cultures that bore names in synonymy and one named culture that did not conform to its original description. All these observations are based upon cultures incubated at 37° to 45° C.

CHARACTERS

**Vegetative rods.*—0.5μ to 0.9μ by 2.5μ to 3.0μ; filaments; resembles *B. subtilis;* cells contain few small fat globules when grown on glucose-nutrient agar; motile; peritrichous flagella; Gram-positive, becoming Gram-variable.

**Sporangia.*—Not definitely swollen; sometimes slight swelling.

**Spores.*—Oval to cylindrical; subterminal to terminal; sporulation better on acid proteose-peptone agar (*105, p. 187*) than on nutrient or glucose-nutrient agar. *Variation:* One strain (No. 796) spores are cylindrical, sometimes bent, and only a few are formed even at 21 days.

Colonies.—Small; round; regular; not distinctive.

Nutrient agar slants.—Growth scant to moderate; thin; flat; rough; off-white.

Glucose-nutrient agar slants.—Growth more abundant than on nutrient agar; smooth; moist. *Variation:* One strain (No. 796) was the same as on nutrient agar.

Proteose-peptone-acid agar slants.—Growth more abundant than on above media; microscopically the cells appear healthier. *Variation:* One strain (No. 796) failed to show increased growth.

Potato.—Growth scant to moderate; thin; spreading; white to cream-colored; potato not digested; may have a sour, vinegarlike odor.

Nutrient broth.—Moderate uniform turbidity, followed by clearing with formation of sediment.

**Glucose broth.*—Heavy growth; pH 4.0 to 4.4 after 7 days; with metallic iron, rapid evolution of gas; none without iron. *Variation:* One strain (No. 796) failed to form gas.

**Voges-Proskauer reaction.*—Positive.

Utilization of citrate.—Negative.

Fermentation tests.—Acid on glucose, fructose, sucrose, maltose, and glycerol; usually no acid on arabinose and sorbitol; no acid on xylose; organic nitrogen preferred to the inorganic.

**Starch hydrolysis.*—Positive.

Nitrites from nitrates.—Usually not formed. *Variation:* Two strains positive.

**Maximum temperatures for growth.*—Majority of strains grow at 54° to 60° C. in water bath. *Variation:* One strain (No. 796) grew poorly at 50° C.

**Gelatin hydrolysis.*—Negative. *Variation:* One strain (No. 609) weakly positive.

**Casein hydrolysis.*—Weakly positive.

TYPE CULTURES STUDIED

The following authentic cultures were received and identified as *Bacillus coagulans.*

Bacillus coagulans

No. 609, from Porter, 1937; Hammer.
Nos. 795, 796,[15] 797, and 798, from Hammer, 1941, his Nos. 195, 196, 198, and 200, respectively.

Bacillus coagulans, from Porter, 1940; Bact. Dept., Iowa State Col. See *B. cereus* No. 833.

SYNONYMY

Named cultures that conform to their descriptions were identified by the writers as *Bacillus coagulans* as follows.

[15] This strain differs from the others in the following characters: The spores are fewer and more cylindrical, sometimes bent; the utilization of glucose is weak as evidenced by lack of better growth in its presence, lack of gas formation from it in presence of metallic iron and lack of formation of acetoin; and no growth occurs above 50°C. The writers believe these characters represent a weak or aberrant strain.

Bacillus dextrolacticus Andersen and Werkman, Iowa State Col. Jour. Sci. 14: 187–193. 1940.

From Werkman, Iowa State Col., 1941 ..... *B. coagulans* No. 784

In describing their new species, Andersen and Werkman noted that their organism was related to *B. coagulans*. Qualitatively, it produced nitrites from nitrates and acid from arabinose and sorbitol, whereas *B. coagulans* did not. Quantitatively, it also produced more acetylmethylcarbinol and more *d* lactic acid from glucose in a shorter time. Culture No. 784 failed to utilize sorbitol in the writers' laboratory, but it did ferment arabinose and reduced nitrates to nitrites. It has not been the policy in this work to make species on such variable characters as these; nor are quantitative characters of any value except in the designation of strains. It is the writers' opinion, therefore, that this is merely a very active strain of *B. coagulans*.

Bacillus thermoacidurans Berry, Jour. Bact. 25: 72–73. 1933.

From NCA, 1940 ..... *B. coagulans* No. 770

Berry named and quite fully described this new species isolated from off-flavor tomato juice but failed to see the similarity between it and the previously established *B. coagulans*. The writers were not able to distinguish between the two.

ASSIGNMENT OF A NAMED CULTURE

The following culture does not conform to its original description and has been identified as *Bacillus coagulans*.

Bacillus modestus Schieblich, Zentbl. f. Bakt. [etc.] Originale (1) 124: 273. 1932.

From Porter, 1940, Schieblich ..... *B. coagulans* No. 905

According to the original account this organism was 1.0μ to 1.2μ in diameter, the protoplasm appeared granular, gelatin was liquefied, and alkali produced in milk. These characters, as well as the illustration showing flagella, might lead one to think the organism was *B. cereus*, certainly not *B. coagulans*. Culture No. 905 is one of the few strains of this species that reduce nitrates to nitrites.

Bacillus firmus

Bacillus firmus Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 470. 1933.

Bacilus firmus was rather difficult to classify because in it are combined some of the morphology of the *B. subtilis* and the *B. circulans–B. brevis* groups and some of the physiology of *B. brevis* and *B. sphaericus*. It is placed here mainly because of its morphology. The rods are quite similar to *B. subtilis* or *B. pumilus*, but the oval spores swell the rod somewhat; not so much, however, as they do in the case of *B. circulans*. In its inability to grow at pH 6.0 or below, it resembles the varieties of *B. sphaericus*.

Eight cultures labeled *B. firmus* were obtained through Porter from Bredemann under whom Werner did his work. Five of them conform to the description given below for *B. firmus*, whereas the other three were strains of *B. brevis*. The original account applies to either species, but since no other name has been given to cultures having the characteristics listed here for *B. firmus*, it is assumed that the five strains are authentic and that some mistake has been made in the case of the three *B. brevis* cultures.

The following description of *B. firmus* is based upon the study of five type cultures, one culture bearing another name, two cultures received without names, and one isolation, a total of nine strains.

CHARACTERS

**Vegetative rods.*—0.6μ to 0.9μ by 1.5μ to 4.0μ; single or in pairs; ends sometimes poorly rounded and almost pointed; motile; Gram-positive.

**Sporangia.*—Usually slightly swollen.

**Spores.*—0.7μ to 0.9μ by 1.0μ to 1.4μ; variable; oval; central to subterminal; usually form within 48 hours; generally more numerous on peptone agar than on nutrient or beef-extract agar.

Colonies.—Small; smooth; round; whitish; not distinctive. *Variation:* One strain (No. 749), pink.

**Nutrient agar slants.*—Growth moderate; flat; opaque; ribbon-like; whitish. *Variation:* One strain, pink.

**Glucose-nutrient agar slants.*—Growth very scant; inhibited by the acid formed from the glucose (pH 6.0).

Potato.—No growth (too acid).

Nutrient broth.—Light uniform turbidity or a flocculent growth in glucose broth, final pH not below 6.0; methylene blue not reduced.

Voges-Proskauer reaction.—Negative; very little if any growth.

Utilization of citrate.—Usually negative.

**Utilization of inorganic nitrogen.*—Negative. *Variation:* One strain uses it slightly.

Fermentation tests.—With organic nitrogen acid from glucose; no acid from arabinose or xylose.

**Starch hydrolysis.*—Positive.

Nitrites from nitrates.—Positive. *Variation:* One strain (No. 749) negative.

Salt tolerance.—Growth in broth containing 4 percent NaCl; some strains even growing in 7 percent salt.

**Urease.*—Not produced.

Maximum temperatures for growth.—Some fail to grow above 37° C., whereas others will grow at 45°.

**Gelatin hydrolysis.*—Positive.

**Casein hydrolysis.*—Positive.

TYPE CULTURES STUDIED

The following type cultures of *Bacillus firmus* were received by the writers.

Bacillus firmus

No. 613, from Porter, 1937; Bredemann, the original strain.
No. 854, from Porter, 1937; Bredemann, strain A; Hamburg.
No. 855, from Porter, 1937; Bredemann, strain B.
No. 858, from Porter, 1937; Bredemann, strain E; Pyramiden.
No. 860, from Porter, 1937; Bredemann, strain G; Dolomiten.

Bacillus firmus, from Porter, 1937; Bredemann, strains C (Rauschen), D (Utliberg), and F (Predigtstuhl). See *B. brevis* Nos. 856-859.

ASSIGNMENT OF A NAMED CULTURE

The following culture differs somewhat from its original description and has been identified by the writers as *Bacillus firmus.*

Bacillus flavidus Stühlrk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 185. 1935.

From Porter, 1940; Bredemann .....*B. firmus* No. 861

Stühlrk stated that starch was not hydrolyzed, nitrites were not formed from nitrates, and gelatin was not liquefied. No. 861, however, is positive in all three characters and, furthermore, its optimum temperature is about 30° C., instead of 38° to 40° as he stated. It agrees, however, with Stühlrk's account as regards the

size of the rods and spores and the lack of growth on potato. Whether this is the original culture or whether the discrepancies are due to different test methods or to variation in the culture cannot be determined.

IDENTIFICATION OF UNNAMED CULTURES

The following two unnamed cultures were identified as *Bacillus firmus.*

Unnamed cultures	*B. firmus* No.
From Broadhurst, Columbia Univ. Teachers Col., 1939	749
From Raper, USDA, 1940, from Myxomycete culture	783

No. 749 is aberrant in that it produces a pink color and fails to reduce nitrates to nitrites.

In addition, one strain of *B. firmus* (No. 769) was isolated by the writers from partially decomposed wheat grain.

Bacillus lentus

Bacillus lentus Gibson, Zentbl. f. Bakt. [etc.] Abt. 2, 92: 368. 1935.

Gibson studied nine strains of *Bacillus lentus* isolated from five different soils and concluded that "they appear to be sufficiently well defined to justify their recognition as a distinct species." He recognized their relation to *B. pasteurii* and the difficulty of classifying this and strains of similar organisms. The present writers had only one strain (Gibson's No. 165) for study. It seems to be distinct from *B. firmus* and to be somewhat related to *B. circulans* (fig. 1). Pending further study of more strains, it is placed near *B. firmus.*

CHARACTERS

**Vegetative rods.*—0.6μ to 0.7μ by 2.0μ to 3.0μ; single or in pairs; few filaments; motile; Gram-positive. On glucose nutrient agar, the cells are swollen and there are many shadow-forms.

**Sporangia.*—Usually slightly swollen.

**Spores.*—0.7μ to 0.8μ by 1.0μ to 1.3μ; central to subterminal.

Colonies.—Small; smooth; round; whitish; not distinctive.

**Nutrient agar slants.*—Growth slow; thin; translucent, becoming opaque; no growth at pH 6.0 or below.

**Glucose-nutrient agar slants.*—Growth scant, inhibited by the acid produced from the glucose (pH 6.0).

Potato.—No growth (too acid).

Nutrient broth.—Light, uniform turbidity.

Voges-Proskauer reaction.—Negative; no growth in glucose broth (slight acidity, pH 6.0 to 6.3, slowly developed, according to Gibson).

Utilization of citrate.—Negative.

Fermentation tests.—No growth on carbohydrate media with ammonia as source of nitrogen; with organic nitrogen, slight acid from arabinose, xylose, and glucose.

**Starch hydrolysis.*—Positive.

Nitrites from nitrates.—Negative.

Salt tolerance.—Growth occurs in nutrient broth containing 4 percent NaCl.

**Urease.*—Produced.

Maximum temperature for growth.—37° C.

**Gelatin hydrolysis.*—Negative.

**Casein hydrolysis.*—Negative.

TYPE CULTURES STUDIED

The following authentic cultures were identified as *Bacillus lentus.*

Bacillus lentus

No. 670, from Gibson, 1937; No. 165.
No. 883, from Porter, 1940; Gibson No. 165.

Group 2. Sporangia Definitely Swollen by Oval Spores

Bacillus polymyxa

Bacillus polymyxa (Prazmowski) Migula, Syst. der Bakt., p. 638. 1900.

Clostridium polymyxa Prazmowski, Inaug. Diss., Leipzig, p. 37. 1880.

Granulobacter polymyxa Beijerinck, K. Akad. van Wetensch. te Amsterdam, 1: sec. 2, No. 10. 1903.

Aerobacillus polymyxa Donker, Inaug. Diss., Delft, p. 138. 1926.

Porter, McCleskey, and Levine (*86*) reviewed the literature and made a detailed study of a large number of cultures of the aerobic sporeformers that produce gas from carbohydrates. They found that their cultures fell into two distinct species, *Bacillus polymyxa* and *B. macerans.* The present writers agree with those findings in all particulars except as to the fermentation of rhamnose and sorbitol. *B. macerans* was said to form acid and gas, whereas *B. polymyxa* did not. All the strains of the latter in the writers' collection, however, produced acid and gas from both substances, and some of them reacted as vigorously as *B. macerans.* Although this is a minor point, an attempt was made to find the reason for the discrepancy in the case of rhamnose. The brand of pure rhamnose recommended by Tilden and Hudson (*106*) was obtained and the test made. The results were the same as before. It is concluded, therefore, that the fermentation of the two substances is a variable character.

The following description of *B. polymyxa* is based upon the study of 11 strains, of which 2 were type cultures, 4 bore names in synonymy, and 5 were isolations from soil.

CHARACTERS

**Vegetative rods.*—0.7μ to 1.0μ by 2.0μ to 7μ; singly or in short chains; on glucose agar cells may or may not be larger and some may contain several small fat globules; motile; Gram-variable.

**Sporangia.*—Distinctly bulged; spindle-shaped or clavate.

**Spores.*—1.0μ to 1.5μ by 1.5μ to 2.5μ; oval; central to terminal; spore wall usually thick and stainable.

Colonies.—Thin; inconspicuous; spreading over entire plate; the rough stage small, round, whitish, and tough.

Nutrient agar slants.—Growth scant to moderate; spreading; indistinct to whitish.

Glucose-nutrient agar slants.—Growth much thicker than on nutrient agar; raised; glistening; gummy; with formation of gas. *Variation:* Some strains do not form gum.

Glucose-nitrate agar slants.—Growth abundant; gummy. *Variation:* One strain showed only a ribbon type of growth.

**Potato.*—Growth moderate to abundant; slimy; whitish to light tan; potato decomposed with formation of gas; growth of rough strains denser and heaped up.

Nutrient broth.—Turbidity uniform to granular, sometimes clear; sediment slimy; final pH of glucose broth cultures 5.2 to 6.5.

**Voges-Proskauer reaction.*—Positive.

Utilization of citrate.—Variable.

**Fermentation tests.*—Acid, gas usually formed from arabinose, rhamnose, xylose, glucose, fructose, galactose, mannose, maltose, sucrose, lactose, raffinose, dextrin, inulin, salicin, glycerol, mannitol, and sorbitol; gum usually formed also.

**Starch hydrolysis.*—Positive; crystalline dextrins not formed.

Nitrites from nitrates.—Positive.

**Methylene blue.*—Reduced, not reoxidized in 21 days.

Maximum temperature for growth.—40° C.

Gelatine hydrolysis.—Positive.

Casein hydrolysis.—Positive.

TYPE CULTURES STUDIED

The following authentic cultures were confirmed as *Bacillus polymyxa*.

Bacillus polymyxa

No. 279, from Christensen, Difco Labs., 1936, No. 8277.
No. 354, from McCoy, Wis. Univ., 1937.

SYNONYMY

The following four cultures that apparently conform to their original descriptions were identified as *Bacillus polymyxa*.

Bacillus asterosporus (Meyer) Migula, Syst. der Bakt., p. 528. 1900.
Astasia asterosporus A. Meyer, Flora 84: 185–248. 1897.

	B. polymyxa No.
From Kral's Collection, Vienna 1921	251
From Christensen, 1936, No. 8278	280
From Porter, 1940; Bredemann	812
From Porter, 1940; Meyer	813

All the above strains were studied in detail and were not distinguishable from *B. polymyxa*, thus confirming the observations of Porter, McCleskey, and Levine (*86*).

ISOLATIONS

Five isolations from soil were made by the writers and were identified as *Bacillus polymyxa*—Nos. 252, 293, 297, 391, and 394.

Bacillus macerans

Bacillus macerans Schardinger, Centbl. f. Bakt. [etc.] Abt. 2, 14: 772–781. 1905.
Rottebazillus 1, Schardinger, Wien. Klin. Wchnschr. 17: 207. 1904.

Owing to its ability to produce acetone, ethyl alcohol, and gas from carbohydrates and its association with the process of retting, *Bacillus macerans* has been studied extensively. Since Porter, McCleskey, and Levine (*86*) have reviewed the literature, it will not be repeated here. They brought out its relation to *B. polymyxa* and gave the characters by which the two could be separated. In the present work, its close relationship to *B. circulans* is also seen. The latter does not produce

gas and, as far as it is known, neither is acetone nor alcohol formed. Although other minor characters serve to differentiate the two, their close relationship is striking. This is further emphasized by the presence of intermediates.

The following description of *B. macerans* is based upon the study of five strains, two of which are type cultures, and the names of the other three are in synonymy.

CHARACTERS

**Vegetative rods.*—0.6μ to 0.8μ by 2.0μ to 8.0μ; singly or in pairs; on glucose-nutrient agar, cells wider and longer than on nutrient agar with a few small fat globules; motile; Gram-variable.

**Sporangia*—Definitely bulged; clavate.

**Spores.*—1.0μ to 1.2μ by 1.5μ to 2.5μ; oval; terminal to subterminal; spore wall thick and often stainable.

Colonies.—Small; thin; transparent to whitish; irregular spreading; rough stage has small compact colonies.

Nutrient agar slants.—Growth moderate; thin; spreading; inconspicuous; with addition of glucose, growth is heavier.

Glucose-nitrate agar slants.—Growth scant.

**Potato.*—Usually no visible growth in 3 days at 28° C.; in 5 to 7 days, growth indistinct, gas formed, and potato decomposed.

Nutrient broth.—Turbidity light, uniform to granular; flocculent sediment; with glucose added, the pH is 5.0 to 5.5 after 7 days.

**Voges-Proskauer reaction.*—Negative.

Utilization of citrate.—Negative.

**Fermentation tests.*—Acid and usually gas from arabinose, rhamnose, xylose, glucose, fructose, galactose, mannose, maltose, sucrose, lactose, raffinose, salicin, inulin, dextrin, glycerol, mannitol, and sorbitol.

**Starch hydrolysis.*—Positive; crystalline dextrins formed.

Nitrites from nitrates.—Positive.

**Methylene blue.*—Reduced and reoxidized in 21 days.

**Maximum temperature for growth.*—Varies from 45° to 50° C., depending upon the strain; slow growth, if any, at 20° C.

Gelatin hydrolysis.—Positive.

Casein hydrolysis.—Negative at 1 week, later becoming weakly positive.

TYPE CULTURES STUDIED

The following authentic cultures were obtained and identified as *Bacillus macerans.*

Bacillus macerans

No. 277, from Christensen, 1936. No. 8275.
No. 888, from Porter, 1940; Schardinger.

SYNONYMY

Named cultures that apparently conform to their descriptions were identified by the writers as *Bacillus macerans.*

Bacillus acetoethylicum Northrup, Ashe, and Senior, Jour. Biol. Chem. 39: 1–21. 1919.

From Christensen, 1936, No. 8276 ..... *B. macerans* No. 278

Bacillus betanigrificans Cameron, Esty, and Williams, Food Res. 1: 73–85. 1936.

	B. macerans No.
From Porter, 1937; Cameron	648
From Cameron, 1937	649

The blackening of canned beets was shown by Cameron and others to depend upon the action of the newly described species in the presence of an abnormally high content of iron in the beet juice. Metallic iron accelerated the growth of the organism, which increased the iron in solution and raised the pH.

When this organism was first studied by the writers (*20, p. 282*) a brownish to black pigment was formed in the presence of metallic iron. In 1942, however, no blackening was obtained either on artificial media or on beets in the presence of metallic iron. In the interval between tests the cultures had been maintained on nutrient agar containing 0.5 percent of starch and kept in a refrigerator. Having lost its original specific characteristic, this species could not be differentiated from *B. macerans*. One might say that this situation is analogous to *B. subtilis* and its black varieties and that the organism under discussion should be *B. macerans* var. *niger*. The writers do not recommend this, unless further study of many more strains should warrant such a separation.

Bacillus circulans

Bacillus circulans Jordan, emend. Ford, Jour. Bact. 1: 519. 1916.
B. circulans Jordan, Mass. State Bd. Health, Expt. Invest., pt. 2, p. 831. 1890.

Bacillus circulans was introduced in 1890 by Jordan, who noticed that the inside of small colonies had a rotary motion, hence the name. Ford isolated a culture in 1916 that he believed to be the same as Jordan's, but a scrutiny of the characteristics as given shows some discrepancies. Jordan gave the size of the rods as 1μ by 2μ to 5μ, having small oval terminal spores that were about the same diameter as the rods; gelatin was liquefied. On the other hand, Ford said his rods were 0.5μ by 2.5μ to 4.0μ, the spores 0.75μ by 1.25μ; the sporangium, therefore, definitely bulged, and gelatin was not liquefied.

Ford's culture shows a rotary motion within the colonies, and if the surface of the agar plate is dried before inoculation, small colonies form at the edge, rotate to the right or left, and move out over the surface (*96*). Although Ford failed to see the liquefaction of gelatin, gelatin is attacked if tested by the Frazier method. The writers believe, therefore, that Ford's culture, the only one of this species available, may be accepted as authentic and that Jordan's measurements and observations on the morphology may have been subject to some errors.

As a result of the study of more than 40 cultures that have the same morphology and a similar physiology but often differing in the macroscopic appearance of the growth, it appears that *B. circulans* is a "parent species" from which has sprung numerous variations. In this respect it is comparable to *B. cereus*, although perhaps showing more variability. Gibson and Topping (*43*) stated that the "*B. circulans* group and the *B. fusiformis* group are each complexes exhibiting variations in several directions . . . ." This is certainly borne out by the present work.

In addition to being quite a variable species, *B. circulans* is connected to its close relatives by intermediates. For instance, *B. macerans* is distinguished from it mainly by the production of gas from carbohydrates and the formation of crystalline dextrins from starch.

But in the writers' collection there are three cultures that do not form gas, although they do produce crystalline dextrins, and two others that form gas but no crystalline dextrins. For the present these cultures are considered intermediates. Another close relative is *B. alvei*, separable from *B. circulans* by a positive V-P reaction and the nonfermentation of arabinose and xylose. Yet in the collection, two cultures are V-P positive and ferment the pentoses. Although *B. macerans* and *B. alvei* are quite distinct from each other, both are closely related to *B. circulans*. *B. polymyxa*, *B. laterosporus*, and, more remotely, *B. firmus* and *B. lentus* are also related to *B. circulans*. In the first instance, this is shown by certain cultures that produce much slime and dissolve casein; in the next, by the adherence of remnants of the sporangium to one side of the spore; and lastly, by the tendency of the spore to swell the sporangium. Because of the variations found in this complex, it has not been easy to define the species. It should be borne in mind that the characteristics studied may not be so important and dependable as some others might be. Furthermore, attempts to induce variation could not be undertaken, as they were in the case of *B. cereus* and *B. subtilis*, but judging from the presence of intermediates it should prove profitable taxonomically.

The following description of *B. circulans* is based upon the study of 44 different strains, of which 3 were type cultures, 2 bore names in synonymy, 14 bore other names, 2 were received unnamed, and 23 were isolations.

CHARACTERS

**Vegetative rods.*—0.5μ to 0.7μ by 2μ to 5μ; sometimes slightly bent; ends rounded or pointed; usually occurring singly; motile, usually exceedingly so; Gram-variable, generally negative. *Variations:* 0.4μ to 0.9μ by 1.5μ to 5.0μ; metachromatic granules; or encapsulated.

On glucose nutrient agar the cells are wider and longer than on nutrient agar; small fat globules easily demonstrated.

**Sporangia.*—Definitely swollen; clavate.

**Spores.*—0.8μ to 1.2μ by 1.1μ to 2.0μ; oval; terminal to subterminal; spore wall thick and stainable. *Variations:* Spores vary greatly in size, even in the same smear, being small or larger than normal; kidney-shaped; central; or remnants of sporangium adhere to one side of spore.

Colonies.—Thin; transparent; spreading over entire surface of plate, sometimes barely visible; rough and mucoid colonies small- to medium-sized, entire, whitish, nonspreading.

**Giant colonies.*—If the surfaces of nutrient agar plates are allowed to dry 2 or 3 days before inoculating with very motile strains, instead of spreading as a thin layer of individual cells, minute rotating colonies proceed out from the edge of the colony, which may become entirely disconnected from it. During the progress of the motile colonies, nonmotile cells are left behind, some of which may develop and help in covering the whole plate.

Nutrient agar slants.—Growth scant, spreading, indistinct. *Variation:* Gummy; adherent; entire; or dense.

Glucose-nutrient agar slants.—Growth thicker than on nutrient

agar. *Variations:* Some strains are yellowish; opaque; very gummy, or encapsulated.

Potato.—Usually no visible growth occurs. *Variations:* Growth moderate to abundant; lemon yellow, pink, brownish; potato darkened; or finely wrinkled.

Nutrient broth.—Turbidity light to fair, with flocculent to slimy sediment. *Variation:* A few strains fail to grow.

**Glucose broth.*—After 7 days pH of cultures generally 5.0 to 5.8.

**Voges-Proskauer reaction.*—Negative.

Utilization of citrate.—Usually negative.

**Fermentation tests.*—Acid without gas on glucose, fructose, mannose, galactose, sucrose, maltose, raffinose, salicin, and dextrin; usually acid from arabinose, xylose, lactose, glycerol, and mannitol; action on rhamnose and inulin variable; one strain does not use inorganic nitrogen.

**Starch hydrolysis.*—Positive.

Nitrites from nitrates.—Variable.

**Methylene blue.*—Reduced; usually reoxidized in a few days. *Variations:* No growth; or not reoxidized in 21 days.

Urease.—A few strains are positive.

Maximum temperatures for growth.—Usually from 40° to 48° C. *Variations:* Few isolations from soil grow poorly at 37°; two strains grow at 52°.

Gelatin hydrolysis.—Positive. *Variations:* Few strains negative.

Casein hydrolysis.—Negative. *Variations:* Eight strains showed a faint and four a good hydrolysis.

TYPE CULTURES STUDIED

The following authentic cultures were obtained and were confirmed as *Bacillus circulans.*

Bacillus circulans

No. 358, from AMNH, 1925; Ford.
No. 726, from ATCC, 1939, No. 4513; Ford No. 26.
No. 831, from Porter, 1940; NCTC No. 2601; Ford No. 26.

Bacillus circulans from Steinhaus. See *B. sphaericus* var. *fusiformis.*

SYNONYMY

Named cultures that apparently conform to their descriptions were identified by the writers as *Bacillus circulans* as follows.

Bacillus closteroides Gray and Thornton, Zentbl. f. Bakt. [etc.] Abt. 2, 73: 93. 1928.

From Porter, 1940; Thornton .....*B. circulans* No. 832

This culture agrees with its original description and with that of *B. circulans* with the possible exception of the utilization of phenol. This property was not investigated in the present work. Judging from the variations found in the utilization of particular carbohydrates, citrates, and casein, it is highly improbable that such a character as the utilization of phenol would be stable or of such importance as to warrant species designation.

Bacillus krzemieniewski Kleczkowska, Norman, and Snieszko, Soil Sci. 49: 185–190. 1940.

From Snieszko, 1942 .....*B. circulans* No. 760

Isolated from arable soil during soil fertility tests by the Azotobacter plaque method, this organism was characterized by the presence of capsules and the production of large quantities of slime, especially on media containing simple carbohydrates and very low in nitrogen. Since it grew well on Ashby's agar

(used for the growth of Azotobacter) it was thought that nitrogen might be fixed, but this could not be demonstrated (*54, p. 189*).

This strain differs from the normal *B. circulans* in being encapsulated and producing gum on carbohydrate media and in the more active decomposition of casein, the latter character being shared with three other strains of *B. circulans*. Because of this, it might possibly be considered as an intermediate between *B. circulans* and *B. polymyxa*. Until more strains are isolated and studied, the writers prefer to consider it as a mucoid stage of growth of *B. circulans*.

ASSIGNMENT OF OTHER NAMED CULTURES

The following 14 cultures do not conform to or are not recognizable from their descriptions and have been identified by the writers as *Bacillus circulans*.

Bacillus carotarum a

From Porter, 1940; Bredemann; Stapp ..... *B. circulans* No. 826

This culture is mislabeled; for authentic culture, see *B. carotarum* in the synonymy of *B. megatherium*.

Bacillus cohaerens

From Porter:	*B. circulans* No.
1940; Bredemann	838A
1940; Stapp	839A

For authentic cultures of this species, see *B. cohaerens* in *B. megatherium–B. cereus* intermediates, group A. Nos. 838A and 839A occurred as contaminants of the original cultures.

Bacillus consolidus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 191. 1935.

From Porter, 1940; Bredemann ..... *B. circulans* No. 841

This is probably not the original culture, although it came from Bredemann's laboratory where Stührk did his work. In the brief account, the spore was said to be round to cylindrical and smaller in diameter than the rods, and the starch was not hydrolyzed, all of which do not apply to No. 841.

Bacillus cubensis Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 192. 1935.

From Porter, 1940; Bredemann ..... *B. circulans* No. 842

This strain is rather weak, grows slowly, forms only a few spores, and requires organic nitrogen. Otherwise it conforms to its characterization. Whether the culture is authentic cannot be stated, because of the lack of pertinent data in the original account.

Bacillus fusiformis

From ATCC:	*B. circulans* No.
1939, No. 61; Ford No. 10	727
1939, No. 4515; Ford No. 24	728

For authentic cultures see *B. sphaericus* var. *fusiformis*. No. 727, a biotype, is interesting because it is the only strain of the species that produced acetylmethylcarbinol. In this respect it shows a relationship to either *B. alvei* or *B. polymyxa* and might be considered an intermediate. Since there is only one strain of this kind, the writers prefer to place it here as a biotype.

Bacillus globigii

From ATCC, 1936, No. 4516; Ford No. 7; Kral ..... *B. circulans* No. 313

For a discussion of cultures hearing the above name, see *B. mesentericus* var. *ruber* in the synonymy of *B. subtilis*.

Bacillus lautus Batchelor, Jour. Bact. 4: 30. 1919.

From Henry, Wash. [State] Univ., 1937, No. 160..... *B. circulans* No. 666

As received, this culture was mixed with *B. cereus*. After purification it checked with Batchelor's description in most respects, but owing to the lack of pertinent data and disagreement with Batchelor's account one cannot be sure that this is the right culture. For instance, the diameter of the rods was given as 1.5μ, the size of *B. megatherium*, which is much larger than No. 666.

Bacillus nitidus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 99. 1935.

From Porter, 1937; Bredemann ..... *B. circulans* No. 625

This strain is rather weak in its fermentation of carbohydrates, which accounts for a higher pH of glucose broth cultures (pH 6.5 after 7 days) and for the lack of reoxidation of methylene blue. It corresponds to the original culture as far as the meager information allows.

Bacillus pabuli Schieblich, Centbl. f. Bakt. [etc.] Abt. 2, 58: 204. 1923.

From Porter, 1940 Schieblich ..... *B. circulans* No. 924

The chief difference noted between this strain and the original as described by Schieblich is in the production of acid from carbohydrates, this culture forming acid readily from carbohydrates (pH glucose broth culture 5.4 after 7 days), whereas Schieblich's culture was said not to do this. Because of the lack of other pertinent data in the original account, it cannot be said that the two strains are identical.

Bacillus supraresistens Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 185. 1935.

From Porter, 1940; Bredemann ..... *B. circulans* No. 977

This strain is somewhat irregular in that it prefers a temperature of about 40° C. and will grow up to 52°, which is 5° to 10° higher than most of the other strains. On potato the growth is yellowish and heaped up like the mucoid strains of this species, but in glucose broth only a little acid is produced; otherwise, it seems to be normal. It probably is authentic.

Bacillus terminalis Migula, Syst. der Bakt., p. 578. 1900.

B. lactis XII Flügge, Ztschr. f. Hyg. u. Infektionskrank. 17: 296. 1894.
B. lacteus Chester, Manual of Determinative Bact., p. 291. 1901.

From ATCC, 1939, No. 4530; Ford No. 28..... *B. circulans* No. 746

Lawrence and Ford (*61, p. 134*) made two isolations which they believed corresponded to this species. No. 746, which came from Ford's collection, conforms to the meager description except that acid is formed in broth containing glucose, lactose, and sucrose, whereas Lawrence and Ford reported alkalinity. It is probably not their culture.

Bacillus validus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 97. 1935.

From Porter, 1937; Bredemann ..... *B. circulans* No. 639

This culture is very similar to No. 625 above, i.e., its ability to ferment carbohydrates is rather weak. Heigener separated them on the basis of the germination of the spore and the reduction of nitrates to nitrites. In this group, especially, neither of these characters can be relied upon.

IDENTIFICATION OF UNNAMED CULTURES

The following two unnamed cultures were received from Anna Russ-Münzer, Hyg. Inst. der Deut. Univ., Prague, and were identified as *Bacillus circulans*.

Unnamed cultures

From Russ-Münzer:	*B. circulans* No.
1938, No. WI	715
1938, No. WII	716

Preliminary observations (*18, p. 496*) allocated No. 715 to *B. alvei*, mainly because of the character and vigor of the growth. There is quite a difference between No. 715 and 716 in this respect, but in physiology they are very close. Russ-Münzer (*89*) was mainly interested in the motility of the growth and noted that similar motility had been seen with *B. circulans* and *B. proteus*. Later, she admitted[16] that the cultures were very much like *B. circulans*, except that gelatin was liquefied. But, as noted above, that is not a definite character unless a delicate method is used to detect changes in the gelatin.

The following 23 strains were isolated from soil by the writers and identified as *B. circulans:* Nos. 291, 292, 294, 295, 359, 375, 381–390, 397–399, and 676–679. Four of these (Nos. 291, 292, 294, and 295) represent the mucoid stage, although

[16] In a letter dated October 22, 1938.

not so extreme as No. 760 (cf. *B. krzemieniewski* in the synonymy). Seven others (Nos. 381–387) did not produce acid so readily in the routine test for carbohydrate fermentation as they should have. In three cases (Nos. 385, 386, and 387), however, the pH of glucose broth after 7 days was from 5.3 to 5.5, which is normal for this species. This indicates, therefore, that some strains do not utilize inorganic nitrogen efficiently.

Bacillus macerans–Bacillus circulans intermediates

It has been pointed out above that *Bacillus macerans* is separated from *B. circulans* mainly by the production of gas from carbohydrates and the formation of crystalline dextrins from starch. It should not be surprising, therefore, if intermediates should be found during the examination of a large number of strains of the two species. Five intermediates are in the present collection. Since there were only five cultures of *B. macerans* studied, it seems doubtful whether this separation represents a natural division. A study of more strains of *B. macerans* is sorely needed.

ASSIGNMENT OF A NAMED CULTURE

The following named culture has been assigned to the *Bacillus macerans-B. circulans* intermediate group.

Bacillus amylolyticus Kellerman and McBeth, Centbl. f. Bakt. [etc.] Abt. 2, 34: 485–494. 1912.

From Kellerman, 1912.....*B. macerans–B. circulans* intermediate No. 290

Kellerman and McBeth isolated and named this organism because of its strong action on starch. At first it dissolved precipitated cellulose (hydroxy-cellulose) with ammonium salts as the source of nitrogen, but after a few years it lost that property. If grown on nutrient broth containing strips of filter paper extending above the surface, it will still usually digest the adhesive substances of the paper, allowing the cellulose fibers to separate and giving the impression that the cellulose is digested. It forms crystalline dextrins but no gas. Morphologically, it is quite aberrant, the spores being cylindrical and swelling the rod only moderately. Only one culture was ever isolated.

Bacillus aporrhocus, recently described by Fuller and Norman (*40, p. 277*), may also belong to this intermediate group. A transplant of it was received too late to be thoroughly studied and assigned. According to the original description, it had motile colonies similar to *B. circulans*. Filter paper was attacked poorly, only slightly more than 3 percent of the cellulose being decomposed in 14 days (*41, p. 284*). When cornstalk cellulose was used instead of filter paper, the decomposition was much greater. Fuller and Norman thought that this may have been due to the presence of cellulosans. Whether this and similar organisms attacking cellulose represent a distinct species or not must remain for future work to decide.

IDENTIFICATION OF UNNAMED CULTURES

The following four unnamed cultures seem to belong to the *Bacillus macerans-Bacillus circulans* intermediate group.

Unnamed cultures

From Meyers, 1939 .....*B. macerans–B. circulans* intermediate No. 765

No. 765 formed crystalline dextrins from starch but no gas from carbohydrates. It was very motile, individual cells moving out from the line of inoculation in plates, thus forming peculiar outgrowths.

Three isolations from soil made by the writers also seem to belong to the *B. macerans–B. circulans* intermediate group, Nos. 373, 374, and 396. The latter produced much slime on carbohydrate media and was similar to the mucoid strains of *B. circulans* (see *B. krzemieniewski* in the synonymy of *B. circulans*), except that crystalline dextrins were formed. Nos. 373 and 374 produced gas from carbohydrates and decomposed potato as *B. macerans*, but no crystalline dextrins were produced from starch, and the maximum temperature allowing growth was about 40° C. In these latter respects, the two strains resembled *B. circulans*.

Bacillus alvei

Bacillus alvei Cheshire and Cheyne, Roy. Micros. Soc. Jour., ser. 2, 5: 592. 1895.

Having been isolated from the hives of honeybees affected with foulbrood, it was thought for a long time that *Bacillus alvei* was the causative agent. In the original description it was noted that the spores, which were very large for the size of the rod, lay parallel and side by side and that the cells grew out from the line of inoculation as pointed processes, the rods growing in a single file or sometimes two or three rods side by side. This latter phenomenon is now known to be the result of the production of motile colonies (*96*), the parallel arrangement of the spores being due to the corresponding position of the rods. Nonmotile variants can be produced in the laboratory (*18*) and probably occur in nature also.

Bacillus alvei is separated from *B. circulans* physiologically, mainly by its formation of acetylmethylcarbinol and its inability to attack the pentoses. It is also closely related to *B. polymyxa.* One culture (No. 395) attacks arabinose and thus shows its relationship to *B. circulans.* It was isolated by the writers and is considered a biotype.

The following description of *B. alvei* is based upon the study of nine strains; of these four were type cultures, two were labeled *B. para-alvei*, and three were isolations by the writers.

CHARACTERS

**Vegetative rods.*—0.5μ to 0.8μ by 2.0μ to 5.0μ; cells frequently lie parallel, side by side; on glucose agar cells contain a few small fat globules; motile; Gram-variable. *Variation:* Nonmotile with capsular material.

**Sporangia.*—Distinctly bulged, spindle-shaped to clavate.

**Spores.*—0.7μ to 1.0μ by 1.5μ to 2.5μ; plump to elongate; central to terminal; free spores frequently maintain a parallel arrangement like the rods.

Colonies.—Thin; smooth; translucent; quickly spreading as a thin layer over the entire plate. *Variation:* Round; raised; entire; or rather gummy.

**Giant colonies.*—If the surfaces of agar plates are dried somewhat by allowing the poured plates to stand at room temperature for 2 days before using, minute colonies containing a few to many cells migrate out from the point of inoculation (*96*). These colonies are usually bullet-shaped and move in large arcs, in contrast to the larger rotating type of colony exhibited by *Bacillus circulans;* plate entirely covered a day after growth starts; variants may not form motile colonies.

Nutrient agar slants.—Growth thin; flat; spreading over the surface; on the upper drier part of the slant, migrating colonies may often be seen. *Variation:* Growth thick; raised; opaque; and rather gummy.

Glucose-nutrient agar slants.—Growth thicker and spreads less than on nutrient agar; slightly gummy.

Potato.—Growth scant to moderate; soft; smooth; spreading; yellowish.

Nutrient broth.—Turbidity light, uniform; some strains produce a pellicle; after 7 days, pH of glucose-broth cultures, 5.0 to 6.0.

**Voges-Proskauer reaction.*—Positive; one strain requires 16 to 20 days' incubation.

Utilization of citrate.—Negative.

**Fermentation tests.*—Acid on glucose, fructose, galactose, sucrose, maltose, dextrin, and glycerol; reaction variable on mannose, lactose, raffinose, salicin, and mannitol; no acid on arabinose, xylose, rhamnose, and inulin.

**Starch hydrolysis.*—Positive.

Nitrites from nitrates.—Negative.

Methylene blue.—Reduced; reoxidation after 21 days variable.

Maximum temperatures for growth.—Varies from 43° to 45° C., depending upon the strain.

Gelatin hydrolysis.—Positive.

Casein hydrolysis.—Positive.

TYPE CULTURES STUDIED

The following type cultures of *Bacillus alvei* were obtained and studied.

Bacillus alvei

No. 662, from Lochhead, Ottawa, 1937.
Nos. 683 and 684, from Hambleton, Bur. Ent. and Plant Quar., Beltsville, Md., 1937, his strains N. C. and Ga. No. 20443.
No. 811, from Porter, 1940; Lochhead No. 127.

Bacillus alvei, from Porter, 1940; Bredemann; Neide. See *B. sphaericus* No. 810.

ASSIGNMENT OF NAMED CULTURES

Named cultures that conform to their original descriptions were identified by the writers as *Bacillus alvei* as follows.

Bacillus para-alvei Burnside, Amer. Bee Jour. 72: 433. 1932.

From Hambleton, 1937, 20485–1 and 20485–2.....*B. alvei* Nos. 685 and 686

This organism was isolated from honeybees affected with parafoulbrood. The bacterial forms found in larvae resembled those found in European foulbrood, which seemed to indicate a close relationship. The distinguishing feature of the bacillus appeared to have been its "marked variability and pleomorphism, depending to some extent on culture medium and temperature but perhaps in greater degree upon its physiological condition" (*10, p. 582*). Coccoid and streptococcic forms were isolated as variants, as well as a small granular rod. The cultures studied by the writers failed to show such morphological variation. They agreed with other strains of *B. alvei*, even producing motile colonies.

ISOLATIONS

Three strains of *Bacillus alvei* were isolated from soil by the writers (Nos. 395, 680, and 750). No. 395 differs from all other strains by being able to utilize arabinose, thus showing its relation to *B. circulans.* It does not use xylose, however. Since there is only one culture, it is considered here as a biotype.

Bacillus laterosporus

Bacillus laterosporus Laubach, Jour. Bact. 1: 511. 1916.

In 1912 White (*116*) gave the name *Bacillus orpheus* to a motile sporeforming organism found in the foulbrood of honeybees and stated that the description would be given later. This was done in 1917 by McCray (*74*). In the meantime, however, Laubach in 1916

described an organism isolated from water, which he called *B. laterosporus* because the spore was formed at the side of the rod, i.e., eccentrically. As the spore matured and became free, a remnant of the wall of the sporangium adhered to one side. This seemed to be quite constant. In the present work, however, certain other closely related species, especially *B. brevis* and *B. circulans*, at times formed spores showing this characteristic, but the number of such spores was never very large. The descriptions of both *B. laterosporus* and *B. orpheus* were quite well done and leave little doubt that they were identical. A comparison of two authentic strains of the former and three strains of the latter confirms this.

The mere naming of a bacterium does not constitute effective publication and is not valid. The name *B. orpheus* should, therefore, be discarded and the name *B. laterosporus* used to designate this species.

The following description of *B. laterosporus* is based upon the study of eight strains, of which five were type cultures and three bore names in synonymy.

CHARACTERS

**Vegetative rods.*—0.5μ to 0.8μ by 2.0μ to 5.0μ; ends poorly rounded or pointed; on glucose agar, cells may contain a few fat globules; motile; Gram-variable.

**Sporangia.*—Definitely bulged; spindle-shaped.

**Spores.*—1.0μ to 1.3μ by 1.2μ to 1.5μ; oval; central to paracentral; lateral; remnants of sporangium adhere to the mature spore, thicker on one side than on the other; sporulation variable, the best medium being potato.

Colonies.—Thin; transparent; irregular; spreading. *Variations:* Small; round; convex; or translucent.

Nutrient agar slants.—Growth moderate; flat; spreading; smooth; translucent to opaque.

Glucose-nutrient agar slants.—Growth heavier than on nutrient agar; sometimes wrinkled at butt.

Potato.—Growth thin; spreading; grayish to pinkish, later becoming brownish.

Nutrient broth.—Turbidity uniform to granular.

**Glucose broth.*—After 7 days, cultures have a pH of 6.0 to 6.4.

**Voges-Proskauer reaction.*—Negative.

Utilization of citrates.—Negative.

Fermentation tests.—Acid from glucose, fructose, maltose, glycerol, and mannitol; reaction variable on galactose, mannose, and salicin; no acid on arabinose, rhamnose, xylose, sucrose, lactose, raffinose, inulin, and dextrin. *Variation:* One strain cannot use inorganic nitrogen.

**Starch hydrolysis.*—Negative.

Nitrites from nitrates.—Positive.

**Methylene blue.*—Reduced and reoxidized within 21 days.

Maximum temperatures for growth.—Varies from 37° to 45° C., depending upon the strain.

Gelatin hydrolysis.—Positive.

Casein hydrolysis.—Positive; some strains weak.

TYPE CULTURES STUDIED

The following type cultures of *Bacillus laterosporus* were obtained and studied.

Bacillus laterosporus

No. 314, from ATCC, 1936, No. 64; AMNH No. 797; Ford No. 6.
No. 340, from NCTC, 1937, No. 2613; Ford No. 29.
No. 347, from ATCC, 1936, No. 4517; Ford No. 29.
No. 590, from NIH, 1937, Clinical Pathological Series.
No. 882, from Porter, 1940; NCTC No. 2613; Ford No. 29.

It should be noted that Ford's No. 29 was obtained from three sources. Numbers 340 and 882 agree quite closely, but No. 347, although agreeing in the main, is a weaker strain. Spores are produced very sparingly, and the culture is not able to use ammonia nitrogen.

SYNONYMY

The following named cultures conform to their original description and have been identified as *Bacillus laterosporus* by the writers.

Bacillus orpheus

	B. laterosporus No.
From Lochhead, 1937	661
From Hambleton, 1937, No. 20485 and No. 2	681 and 682

Bacillus brevis

Bacillus brevis (Flügge) Migula, emend. Ford, Jour. Bact. 1: 522. 1916.

B. brevis (Flügge) Migula, Syst. der Bakt., p. 583. 1900.

B. lactis I, Flügge, Ztschr. f. Hyg. u. Infektionskrank. 17: 294. 1894.

In 1900, Migula renamed Flügge's *Bacillus lactis* I, calling it *B. brevis*, but added nothing to the original account. In 1904, Neide changed the name of Flügge's culture to *B. lactis* and added considerably to its characterization. Cultures of *B. lactis* are still available that conform in general to Neide's description (*81*). Such cultures were studied in the present work and are listed in the synonymy of *B. cereus*. Ford isolated three organisms from soil and called them *B. brevis*, because he believed that they fitted Flügge's and Migula's accounts. He described them fully and deposited cultures with the NCTC and the ATCC. Unfortunately, the strains from the latter do not correspond to Ford's description, being strains of *B. pumilus*. The culture from the NCTC, on the other hand, does agree and is accepted as a true representative of Ford's cultures. So far as the writers are aware, there is no culture available from Migula or Flügge that bears this name and, therefore, no ambiguity or confusion exists between *B. lactis* and *B. brevis*.

The name *B. brevis* is evidently established abroad (*43*) as well as in America, where it has recently received a great deal of publicity from the work of Dubos and associates (*33*). Dubos isolated a soil bacillus that produced antibiotic substances (gramicidin, tyrothrycin). It was identified by the writers and placed in the collection as *B. brevis* No. 751. Several other stock cultures sent to Dubos were tested in his laboratory and were found to produce small quantities of antibiotics but not nearly so much as his isolations.[17] This was

[17] Oral communication, 1940.

expected, as Dubos had specially selected his cultures for this particular character over a period of 2 years.

The following description of *B. brevis* is based upon a study of 40 strains; of these 1 was the type culture, 1 bore a name in synonymy, 25 bore other names, 7 were received unnamed, and 6 were isolated from soil by the writers.

CHARACTERS

**Vegetative rods.*—0.4μ to 0.8μ by 1.5μ to 5.0μ; ends poorly rounded or pointed, occurring singly or paired; on glucose agar, cells usually larger and contain numerous small fat globules; two strains encapsulated; motile; Gram-variable, usually negative.

**Sporangia.*—Definitely bulged; spindle-shaped to clavate.

**Spores.*—1.0μ to 1.3μ by 1.5μ to 2.0μ; oval; central to terminal; spore wall usually thick and stainable; occasional culture may have a few spores with remnants of the sporangia adhering to one side, thus showing the close relation of this species to *Bacillus laterosporus*.

Colonies.—Thin; smooth; translucent; quickly spreading over entire plate. *Variation:* Small, round, nonspreading.

Nutrient agar slants.—Growth thin; smooth; spreading; translucent, becoming opaque and creamy with age. *Variation:* Growth remains thin and does not spread.

Glucose-nutrient agar slants.—Growth more abundant, often wrinkled toward the bottom of the slant.

Potato.—Growth scant to moderate; flat; spreading; watery; creamy yellow to brownish; potato darkened; frequently cannot be distinguished from the growth of *B. pumilus*. *Variations:* More pigmentation, pink to lemon yellow.

Nutrient broth.—Turbidity usually heavy, uniform, with or without a fragile pellicle.

**Glucose broth.*—After 7 days, pH is 8.0 to 8.6; one isolation, pH 6.8.

**Voges-Proskauer reaction.*—Negative.

Utilization of citrates.—Positive. *Variation:* Five strains negative.

**Fermentation tests.*—Acid on glucose, fructose, maltose, and sucrose; usually acid on galactose and glycerol; reaction variable on rhamnose, xylose, mannose, lactose, raffinose, inulin, dextrin, and salicin. *Variation:* Four strains cannot use inorganic nitrogen; on media containing peptone, acid formation, if any, is masked by the alkalinity produced by the strong proteolytic activity of the organism.

**Starch hydrolysis.*—Negative.

**Methylene blue.*—Reduced; not reoxidized in 21 days.

Nitrites from nitrates.—Variable.

Maximum temperatures for growth.—Usually between 45° C. and 48°. *Variations:* Four strains will grow at 50°; four at 52°, and two at 54°.

Gelatin hydrolysis.—Positive.

Casein hydrolysis.—Positive.

TYPE CULTURE STUDIED

The following type culture of *Bacillus brevis* was obtained and studied.

Bacillus brevis

No. 604, from Porter, 1937; NCTC No. 2611; Ford No. 27B.

Bacillus brevis

From ATCC, 1939, No. 18; AMNH; Ford. *See B. pumilus* 724.
From ATCC, 1939, No. 4510; Ford No. 27. See *B. pumilus* 725.

SYNONYMY

A named culture that conforms to its description was identified as ***Bacillus brevis.***

Bacillus centrosporus Ford, Jour. Bact. 1: 524. 1916.

From Henry, Wash. [State] Univ., 1937, No. 120.....*B. brevis* No. 664

According to Ford, the spores were central and the growth did not spread. These facts could not be confirmed with the above culture; the spores were central to terminal and the growth spreading. Since similar variations were found in other strains of *B. brevis*, No. 664 may be considered authentic.

ASSIGNMENT OF OTHER NAMED CULTURES

The following named cultures that do not conform to or are not recognizable from their original description were identified as *Bacillus brevis.*

Bacillus aegyptiacus Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 459. 1933.

From Porter, 1940; Bredemann .....*B. brevis* No. 803

Bacillus alpinus Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 465. 1933.

From Porter, 1937; Bredemann .....*B. brevis* No. 603

Bacillus bellus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 96. 1935.

From Porter, 1937; Bredemann .....*B. brevis* No. 605

Bacillus betainovorans Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 94. 1935.

From Porter, 1937; Bredemann .....*B. brevis* No. 606

Bacillus borstelensis Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 179. 1935.

From Porter, 1940; Bredemann .....*B. brevis* No. 818

This culture does not utilize inorganic nitrogen.

Bacillus dentatus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 106. 1935.

From Porter, 1937; Bredemann .....*B. brevis* No. 611

Bacillus elegans Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 103. 1935.

From Porter, 1937; Bredemann .....*B. brevis* No. 612

The above-named cultures were so poorly described by various workers in Bredemann's laboratory that it is not possible to say whether these represent the original cultures.

Bacillus firmus

From Porter:	*B. brevis* No.
1940; Bredemann, strain Rauschen	856
1940; Bredemann, strain Utliberg	857
1940; Bredemann, strain Predigtstuhl	859

Seven cultures bearing this name were received, four of which are considered authentic and are treated under the species *B. firmus.*

Bacillus formosus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 101. 1935.

From Porter, 1937; Bredemann .....*B. brevis* No. 614

Bacillus granularis Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 180. 1935.

From Porter, 1940; Bredemann .....*B. brevis* No. 870

These two species (*B. formosus* and *B. granularis*) were very meagerly described.

Bacillus hollandicus Stapp, Centbl. f. Bakt. [etc.] Abt. 2, 51: 47. 1920.

From Porter:	*B. brevis* No.
1937; Bredemann	616
1940; Stapp	874

In general these two cultures (Nos. 616, 874) agree with Stapp's publication, minor differences, as a slightly larger diameter of the spores and the reduction of methylene blue, being noted. Stapp gave the maximum temperature as 56° to 59° C., whereas it was 52° to 54° in the writers' test. Differences in technique might account for all these discrepancies. It might be mentioned here that the maximum temperature varies greatly among various strains of this species.

Bacillus limnophilus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 190. 1935.

From Porter, 1940; Bredemann .....*B. brevis* No. 887

This strain was not able to use mineral nitrogen.

Bacillus maculatus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 184. 1935.

From Porter, 1940; Bredemann .....*B. brevis* No. 889

Bacillus montanus Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 458. 1933.

From Porter, 1937; Bredemann .....*B. brevis* No. 622

These (Nos. 622, 887, 889) were also inadequately described.

Bacillus mycoides

From Porter, 1940; Bredemann; Gottheil .....*B. brevis* No. 910

For authentic strains, see *B. cereus* var. *mycoides*.

Bacillus pallidus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 98. 1935.

From Porter, 1937; Bredemann .....*B. brevis* No. 628

Bacillus rarus Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 456. 1933.

From Porter, 1937; Bredemann .....*B. brevis* No. 632

The above two cultures may or may not be the original ones.

Bacillus rufescens Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 178. 1935.

From Porter, 1940; Bredemann .....*B. brevis* No. 948

This strain produces a reddish-brown discoloration of most media and does not use mineral nitrogen. The pigmentation is increased to a dark red or black if tyrosine is added to nutrient agar. There is a possibility that this strain should be considered a variety, such a decision awaiting further information and the isolation of more strains (cf. *B. subtilis* var. *niger* and var. *aterrimus*).

Bacillus santiagensis Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 188. 1935.

From Porter, 1940; Bredemann .....*B. brevis* No. 953

Inorganic nitrogen is not utilized by this culture.

Bacillus segetalis Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 467. 1933.

From Porter, 1937; Bredemann .....*B. brevis* No. 635

Bacillus valinovorans Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 104. 1935.

From Porter, 1937; Bredemann .....*B. brevis* No. 640

It cannot be determined from their descriptions whether any of the above three cultures represent the originals.

Bacillus ventricosus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 102. 1935.

From Porter, 1937; Bredemann .....*B. brevis* No. 641

In 1902, Weiss (*114, p. 233*), described a species under this name that was apparently quite different from the above. Under the rules of nomenclature, Heigener's use of the same name is not allowed.

IDENTIFICATION OF UNNAMED CULTURES

The following unnamed cultures were identified as *Bacillus brevis* during the course of the work.

Unnamed cultures	*B. brevis* No.
From Dubos, 1939, from soil	751
From Dubos, 1940, from Oka cheese	779
From Dubos, 1940, from Turkish cheese	780
From Dubos, 1940, from sewage	781
From Lamanna, Wash. [State] Col., 1941, No. B40	799
From Bondi, Temple Univ., 1942, Collabella strain	1027
From Bondi, Temple Univ., 1942, Schotte strain	1028

Six strains of *B. brevis* were isolated from soil by the writers (Nos. 376–380, and 761). One of these (No. 379) was atypical in that the pH of glucose proteopeptone broth cultures after 7 days was only 6.8 instead of 8.0 or above. In this respect it resembled *B. laterosporus* and was placed in table 13 as an intermediate. It is probably only a biotype.

Group 3. Sporangia Swollen by Round Spores

Bacillus sphaericus

Bacillus sphaericus Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 350. 1904.

In 1904, Neide gave the name of *Bacillus sphaericus* to a round, terminal-spored organism. Ford (*60, p. 520*) considered it identical with *B. pseudotetanicus* (Kruse) Migula (*77, p. 626*) and, because of priority, he recommended the use of the latter name. Kruse's culture, however, was said to be aerobic at ordinary temperature but produced spores only at higher temperatures and under anaerobic conditions. There is, therefore, some doubt as to whether they were the same. The situation is further complicated by the fact that Migula gave the name *B. pseudotetani* to an anaerobic gas-forming organism (*77, p. 598*). Apparently both names have been applied to the aerobic organism. The writers recommend, therefore, that *B. pseudotetanicus* and *B. pseudotetani* be considered nomina dubia and that *B. sphaericus* be nomen conservandum.

Bacillus sphaericus is quite inactive physiologically, its negative characters and its morphology determining its identity for the most part. There seem to be three varieties that are very closely related to it. This arrangement may have to be changed, however, if more strains are isolated or if studies on dissociation are made.

The following description of *B. sphaericus* is based upon 4 type cultures, 2 cultures bearing names in synonymy, 3 bearing other names, 3 isolations from soil and 1 isolation from the intestine of an angleworm, a total of 13 strains.

CHARACTERS

**Vegetative rods.*—0.6μ to 1.0μ by 1.0μ to 7.0μ; ends round to pointed; on glucose agar, cells contain few small fat globules; motile; Gram-variable, often negative with positive granules.

**Sporangia.*—Definitely bulged, mostly clavate (drumstick); some spindle-forms.

**Spores.*—0.7μ to 1.3μ in diameter; round, terminal to subterminal; spore wall usually thick; remnants of sporangium may adhere, sometimes making the surface quite rough; immature spores oval, becoming round or very nearly so; sporulation variable, some strains forming only a few spores.

Colonies.—Thin; smooth; translucent; spreading; entire plate often quickly covered; less strongly motile strains, small, round, raised, edges irregular.

**Giant colonies.*—On agar plates that are allowed to stand 1 or 2 days before using, small motile colonies swarm out from the edge of the inoculum in the case of 7 of the 13 strains (cf. *B. circulans* and *B. alvei*).

Nutrient agar slants.—Growth thin; smooth; spreading; transparent to opaque. In rough stage, growth does not spread and is slightly wrinkled. Growth is the same on glucose-nutrient agar slants.

Potato.—Growth thin; soft; spreading: gray, usually becoming yellowish brown with age.

Nutrient broth.—Turbidity uniform; rough stage, turbidity granular; pellicle membranous.

**Glucose broth.*—After 7 days, cultures have a pH of 8.3 to 8.6.

**Voges-Proskauer reaction.*—Negative.

Utilization of citrates.—Negative. *Variation:* Five strains positive.

**Fermentation tests.*—No acid formed.

**Starch hydrolysis.*—Negative.

Nitrites from nitrates.—Not formed.

Methylene blue.—Reduced; not reoxidized in 21 days.

**Salt tolerance.*—Growth occurs in broth containing 4 percent NaCl.

**Urease.*—Not produced.

Maximum temperatures for growth.—Varies from 40° to 45° C., depending upon the strain.

Gelatin hydrolysis.—Positive; change very slow with some strains.

Casein hydrolysis.—Negative. *Variation:* Three strains show weak action.

TYPE CULTURES STUDIED

The following type cultures of *Bacillus sphaericus* were studied.

Bacillus sphaericus

No. 344, from ATCC, 1936, No. 94; AMNH No. 471; Ford.
No. 348, from ATCC, 1936, No. 4525; Ford No. 25.
No. 966, from Porter, 1940; Claussen 1911.
No. 967, from Porter, 1940; Claussen 1913.

Bacillus sphaericus

From Porter, 1940; Bredemann. See *B. subtilis* No. 963.
From Porter, 1940; Stapp. See *B. megatherium–B. cereus* intermediates, group B.
From Porter, 1940; Edwards (Ford No. 25). See *B. megatherium–B. cereus* intermediates, group B.

SYNONYMY

Named strains that conform to their original descriptions were identified as *Bacillus sphaericus* as follows.

Bacillus lactimorbus Jordan and Harris, Amer. Med. Assoc. Jour. 50: 1669. 1908.

From ATCC, 1939, No. 245 ..... *B. sphaericus* No. 732

Jordan and Harris (*52*) isolated this from the viscera of two heifers and a horse affected with milk sickness or trembles, calling it *B. lactimorbi.* It was weakly pathogenic and was thought to be the causative agent. Later, Sackett (*90*) refuted this, showing that the cause of the disease was the eating of a poisonous plant. The original account applies to the present culture, although spore formation is now slow.

Bacillus lactimorbus, from ATCC, 1939, No. 246; Jordan. See *B. cereus* No. 733.

Bacillus serositidis Lacorte. Inst. Oswaldo Cruz. Mem. 26: 1–9. 1932. Eng. summary, p. 10.

From Lacorte, Brazil, 1937 ..... *B. sphaericus* No. 668

ASSIGNMENT OF OTHER NAMED CULTURES

The following named cultures did not conform to their original description and have been identified as *Bacillus sphaericus*.

Bacillus alvei

From Porter, 1940; Bredemann; Neide ..... *B. sphaericus* No. 810

Obviously this culture was misnamed; it bears no resemblance to *B. alvei*.

Bacillus pseudotetani (Tavel) Migula, Syst. der Bakt., p. 598. 1900.
Pseudotetanus bacillus, Tavel, Centbl. f. Bakt. [etc.] Originale (1) 23: 538. 1898.

From Tittsler, U. S. Bur. Dairy Indus., 1939..... *B. sphaericus* No. 719

Migula said this organism was an anaerobe, had oval spores, and produced more gas than the tetanus bacillus. This culture is, therefore, not authentic. See the discussion of *B. sphaericus* above.

Bacillus pseudotetanicus, from Porter, 1940; NCTC No. 2609; Ford No. 25. See *B. pumilus* No. 937.

Bacillus simplex

From ATCC, No. 4526, 1936 ..... *B. sphaericus* No. 349

See *B. megatherium–B. cereus* intermediates, group C, for the allocation of cultures bearing this name.

ISOLATIONS

Three strains of *Bacillus sphaericus* (Nos. 250, 400, and 752) were isolated from soil; another (No. 717) from the intestine of an angleworm.

Bacillus sphaericus var. *rotans*

Bacillus sphaericus var. *rotans*, new combination.
B. rotans Roberts, Jour. Bact. 29: 234. 1935.

Roberts was the first to see and properly evaluate such motile colonies as are produced by *Bacillus sphaericus* var. *rotans*. Later, Smith and Clark (*96*) noted this phenomenon in cultures of *B. alvei*, *B. circulans*, and an unidentified Gram-negative rod. In the present work, about half of the strains of *B. sphaericus* showed this characteristic. Motile colonies are not, therefore, limited to one species or to a single genus.

Unfortunately, only one strain was available for study, Roberts having lost a considerable number of isolations obtained from the intestines of termites and from soil.[18] The writers have placed it here as a variety, pending the isolation and study of more strains. It differs from *B. sphaericus* in the following characters.

CHARACTERS

Nutrient agar slant.—No growth at pH 6.0 or below.
Nutrient broth.—No growth in broth containing 4 percent NaCl.
Maximum temperature for growth.—35° C.

[18] Oral communication, 1941.

TYPE CULTURE STUDIED

The following type culture was studied and is now designated as *Bacillus sphaericus* var. *rotans*.

Bacillus rotans No. 633, from Porter, 1937; Lewis; Roberts.

Bacillus sphaericus var. *fusiformis*

Bacillus sphaericus var. *fusiformis*, new combination.
B. fusiformis Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 724. 1901.

Gottheil introduced this organism in 1901, thus antedating Neide's *Bacillus sphaericus* by 3 years. The descriptions of both species were practically the same, there being a slight difference in the size of the spores and in the liquefaction of gelatin. Lawrence and Ford (*61, p. 312*) considered *B. fusiformis* identical with *B. lactimorbus*. (See synonymy of *B. sphaericus* above.) They made no mention of urease production.

In the present work, it was found that the only difference between *B. sphaericus* and *B. fusiformis* is the production of urease by the latter. The similarity extends even to the production of motile colonies. It would appear, then, that *B. fusiformis* is a variety or biotype of *B. sphaericus*. The writers have chosen, for the present, to consider it as a variety, realizing that more work on this group and on the production of urease is needed.

It should be mentioned here that the selection of the name *B. fusiformis* by Gottheil in 1901 was unfortunate, owing to the existence of *B. fusiformis* Viellon and Zuber (*112, p. 540–541*). Because of this and the reasons given above, it is recommended that hereafter *B. fusiformis* be known as *B. sphaericus* var. *fusiformis*. Its description is the same as for *B. sphaericus* with the following addition.

CHARACTER

Urease.—Produced.

TYPE CULTURE STUDIED

The following type cultures were studied and are now designated as *Bacillus sphaericus* var. *fusiformis*.

Bacillus fusiformis

No. 339, from NCTC, 1937, No. 2608. Ford No. 24D.
No. 350, from AMNH, 1920, No. 732. (This was mixed with *B. pumilus* No. 247.)
No. 866, from Porter, 1940; NCTC No. 2608; Ford No. 24D.

Bacillus fusiformis

From ATCC, 1939, No. 61; Ford No. 10. See *B. circulans* No. 727.
From ATCC, 1939, No. 4515; Ford No. 24. See *B. circulans* No. 728.
From Porter, 1940; Bredemann. See *B. pumilus* No. 864.
From Porter, 1940; Stapp. See *B. pumilus* No. 865.
From Porter, 1940; Claussen. See *B. cereus* No. 867.

ASSIGNMENT OF A NAMED CULTURE

The following named culture was identified as *Bacillus sphaericus* var. *fusiformis*.

Bacillus circulans

From Steinhaus, USPHS, 1942.....*B. sphaericus* var. *fusiformis* No. 1023

This was isolated by Steinhaus (*104, pp. 765, 782*) from the alimentary canal of the Cecropia moth larva (*Samia cecropia* (L.)) and not identified correctly.

IDENTIFICATION OF UNNAMED CULTURES

The following unnamed cultures were received and were identified by the writers as *Bacillus sphaericus* var. *fusiformis*.

Unnamed cultures	*B. sphaericus* var. *fusiformis* No.
From NIH, 1937	592, 593
From Lamanna, Wash. [State] Col., 1941, No. A20	800

One culture (No. 718) was isolated from soil by the writers.

Bacillus sphaericus var. *loehnisii*

Bacillus sphaericus var. *loehnisii*, new combination.
B. loehnisii Gibson, Jour. Bact. 29: 495. 1935.

Gibson considered *Bacillus loehnisii* very close to *B. pasteurii*, which requires urea for growth, differing from it by the ability to grow in ordinary neutral media. In that case, according to the standards of the present paper, it would be a variety of *B. pasteurii*. The writers have preferred to view it from the opposite direction, as a strain of *B. sphaericus* that had acquired a preference for ammonia and the ability to form urease. It differs from *B. sphaericus* in the following characters.

CHARACTERS

Nutrient agar slants.—No growth at pH 6.0 or below.
Urease.—Produced.

TYPE CULTURE STUDIED

The following type culture was studied.

Bacillus loehnisii

No. 672, from Gibson No. 97, 1937.

Only one strain was studied. It is, therefore, quite possible that with additional strains one might wish to make another allocation. The present arrangement seems logical, although it might seem to be a biotype instead of variety.

Bacillus pasteurii

Bacillus pasteurii (Miquel) Migula, Syst. der Bakt. p. 726. 1900.
Urobacillus pasteurii Miquel, Ann. de Micrographie 1: 552. 1889; 2: 13. 1890.

Bacillus pasteurii was recognized by Löhnis and Kuntze (*72*) because of its ability to decompose urea rapidly. More recently it has been studied further by Gibson (*42, p. 491*). One character separates it from the other members of this round-spored group, and that is its inability to grow in the absence of urea or free ammonia.

The following description of *B. pasteurii* is based on the study of three strains. Since no growth occurs in ordinary media, 1 percent urea was added to all media. It was sterilized by filtration and added aseptically in appropriate quantities.

CHARACTERS

**Vegetative rods.*—0.6μ to 0.8μ by 1.5μ to 2.0μ; occurring singly, sometimes in chains; ends rounded; motile; Gram-variable.

**Sporangia.*—Bulged, sometimes only slightly so.
**Spores.*—Round, 1.0μ to 1.2μ in diameter; terminal to subterminal; young spores may be ovoid.
Colonies.—Very small, not distinctive.
Nutrient agar slants.—Growth thin; transparent to translucent; very little spreading; no growth at pH 6.0 or below.
Nutrient broth.—Turbidity moderate to heavy, uniform.
Voges-Proskauer reaction.—Negative.
**Fermentation tests.*—No acid from carbohydrates.
**Starch hydrolysis.*—Negative.
Nitrites from nitrates.—Positive.
**Salt tolerance.*—Will grow in broth containing 4 percent NaCl.
**Urease.*—Produced.
Maximum temperatures for growth.—Varies from 35° to 40° C., depending upon the strain.
Gelatin hydrolysis.—Positive.
Casein hydrolysis.—Two strains negative, one weakly positive.

TYPE CULTURES STUDIED

The following type cultures were studied.

Bacillus pasteurii

No. 673, from Gibson, No. 22, 1937.
No. 674, from Löhnis, Leipzig, 1914; Kral.
No. 675, from Löhnis, isolation HAI, 1914.

UNCLASSIFIED CULTURES

Four cultures bearing species names were received and studied that have not been placed in any group, owing to a lack of time for more detailed study. In every case, the species is represented by only the single culture. Whether they are aberrant or intermediate forms of established species or whether they represent species that have not been found more than the one time, is difficult to say. The former seems the more likely. A discussion of the available data on the four species follows.

Bacillus aminovorans

Bacillus aminovorans Dooren de Jong, Zentbl. f. Bakt. [etc.] Abt. 2, 71: 215. 1927.

Dooren de Jong isolated *Bacillus aminovorans* from an enrichment culture of garden soil in a mineral solution containing trimethylamine. It was very briefly described and was said not to attack glucose, although in one table a positive reading was recorded. It was relatively inactive on the amines except on methylamine.

TYPE CULTURE STUDIED

The following authentic culture was studied.

Bacillus aminovorans

No. 341, from NCTC, 1937, No. 2870; Dooren de Jong.

It is a Gram-positive large rod (1.1μ to 1.6μ by 2.0μ to 5.0μ), having a granular appearance and often resembling *B. megatherium*. The spores are round or nearly so and do not swell the sporangium appreciably. Scant growth occurs on ordinary media unless an amine is added (tyrosine, cystine, alanine). With

ammonia nitrogen, glucose is the only carbohydrate utilized; starch is hydrolyzed in nutrient agar. Its maximum temperature is about 37° C. Urease is produced; gelatin is weakly attacked, but casein is not.

The data suggest that this may be an aberrant strain of *B. megatherium*, a culture that has become quite restricted in its physiology. Spores that are nearly round have been seen in cultures of *B. megatherium*, and in the present culture they may result from an exaggerated development of that tendency.

Bacillus badius

Bacillus badius Batchelor, Jour. Bact. 4: 25. 1919.

Bacillus badius was obtained from a child's feces. The growth was frequently arborescent, suggesting *B. mycoides*, but physiologically it differed considerably.

TYPE CULTURE STUDIED

The following authentic culture was studied.

Bacillus badius

No. 663, from Henry, Wash. [State] Univ., 1937, No. 110.

This culture seems to agree with the original description. Morphologically it is quite similar to a partially dissociated culture of *B. cereus* var. *mycoides*, or to a large-celled *B. subtilis*. The type of growth suggests the former, but its maximum temperature is 52° C., which indicates the latter. Carbohydrates are not attacked in the presence of organic or inorganic nitrogen. Gelatin and casein are readily hydrolized. No allocation is suggested; more cultures and further work are needed.

Bacillus freudenreichii

Bacillus freudenreichii (Miquel) Migula, Syst. der Bakt., p. 726. 1900.

Urobacillus freudenreichii Miquel, Ann. de Micrographie, 2: 367, 488. 1890; Miquel and Cambier, Traite de Bact., p. 578. 1902.

Gibson (*42, p. 493*) reviewed the literature on *Bacillus freudenreichii* and decided it belonged to the *B. pasteurii* group. No authentic culture was available.

TYPE CULTURE STUDIED

The following type culture was studied.

Bacillus freudenreichii

No. 671, from Gibson, No. 68, 1937.

Morphologically this was similar to *B. pumilus* that has lost the ability to ferment carbohydrates and has developed the production of urease. If any considerable number of such strains are found hereafter, provision for a variety or even a separate species could be made. But since only one strain is at hand and that one not authentic, it is hardly feasible to allocate it.

Bacillus rarerepertus

Bacillus rarerepertus Schieblich, Zentbl. f. Bakt. [etc.] Originale (1), 124: 274. 1932.

This isolation, *Bacillus rarerepertus*, from turnip leaves was said to grow better at 37° C. than at room temperature and to grow on all ordinary media. The rods were 1.1μ to 1.5μ by 1.2μ to 4.5μ and developed round spores.

TYPE CULTURE STUDIED

The following type culture was studied.

Bacillus rarerepertus

No. 943, from Porter, 1940; Schieblich.

If this is the original culture, a great reduction in the size of the rods has taken place. They are now 0.6μ to 0.7μ in diameter and 2.0μ or more in length. Spores are 1.0μ by 1.5μ. Growth is as good at 28° C. as at 37°, spores are formed at the former but not at the latter temperature. Except for its inability to attack carbohydrates, it might be called a weakened culture of *B. circulans*. There is considerable doubt whether this is identical with the original isolation.

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INDEX TO MESOPHILIC MEMBERS OF THE GENUS BACILLUS

The following index to named cultures studied is presented to facilitate finding in this paper the disposition that has been made of these cultures. If it appeared certain that the culture did not represent the organism originally described under that name, its identification is placed in parentheses. Some not in parentheses also may not be the original. For instance, *Bacillus aegyptiacus*, *B. alpinus*, and others were so poorly described that a definite statement of their authenticity cannot be made. Another example is *B. luteus;* of three cultures bearing that name one was identified as *B. cereus*, one as *B. subtilis*, and the other as *B. pumilus*. Which, if any, of these is the true *B. luteus* cannot be determined. A named culture may appear in the synonymy of the species under which it has been placed or in the list of cultures assigned to that species. NUMERALS refer to the number of the culture in the writers' collection.

Name as received	Identification
acetoethylicum	*macerans* 278
adhaerens Laubach	(*pumilus* 309, 337)
adhaerens Stührk	*subtilis* 802
aegyptiacus	*brevis* 803
aethylicus	*cereus* 804
agrestis	*megatherium–cereus* intermediate group A, 602
agri	*cereus* 310, 342; *pumilus* 336
agrophilus	*pumilus* 807
albolactis	*cereus* 721, 808
alpinus	*brevis* 603
alvei	*alvei* 662, 683, 684, 811; (*sphaericus* 810)
aminovorans	Unclassified 341
amylolyticus	*macerans–circulans* intermediate 290
anthracis	*cereus* var. *anthracis* 1007, 1008, 1009, 1010, 1011, 1012, 1013, 1014, 1015, 1016, 1017, 1018, 1019, 1020
asterosporus	*polymyxa* 251, 280, 812, 813
aterrimus	*subtilis* var. *aterrimus* 653; (*pumilus* 266; *megatherium* 267; *subtilis* 263, 330, 652, 814; *cereus* 722, 723)
badius	Unclassified 663
bellus	*brevis* 605
betainovorans	*brevis* 606
betanigrificans	*macerans* 648, 649
borstelensis	*brevis* 818
brevis	*brevis* 604; (*pumilus* 724, 725)
butyricus	*subtilis* 820
calidolactis	(*subtilis* 821)
capri	*megatherium* 607, 822, 824
carotarum	*megatherium* 608, 828, 829; (*circulans* 826; *pumilus* 827)
centrosporus	*brevis* 664
cereus	*cereus* 232, 305
cereus var. *fluorescens*	*cereus* 645
circulans	*circulans* 358, 726, 831; (*sphaericus* var. *fusiformis* 1023)
closteroides	*circulans* 832
coagulans	*coagulans* 609, 795, 796, 797, 798; (*cereus* 833)
cobayae	*megatherium* 610, 835, 837
cohaerens	*megatherium–cereus* intermediate group A, 838B. 839B; (*pumilus* 334; *circulans* 838A, 839A)

Name as received	Identification
consolidus	*circulans* 841
cubensis	*circulans* 842
danicus	*megatherium* 245, 246; (*subtilis* 601)
dendroides	*pumilus* 844
dentatus	*brevis* 611
dextrolacticus	*coagulans* 784
elegans	*brevis* 612
ellenbachensis	*cereus* 847, 848, 849, 850, 851
fastidiosus	(*pumilus* 643)
filiformis	*subtilis* 778
firmus	*firmus* 613, 854, 855, 858, 860; (*brevis* 856, 857, 859)
flavidus	*firmus* 861
mesentericus–flavus (*flavus*)	*pumilus* 333, 739
flexus	*megatherium–cereus* intermediate group A, 665
formosus	*brevis* 614
freudenreichii	Unclassified 671
fusiformis	*sphaericus* var. *fusiformis* 339, 350, 866; (*circulans* 727, 728; *pumilus* 247, 864, 865; *cereus* 867)
geniculatus	*subtilis* 772
globigii	(*pumilus* 331; *circulans* 313)
goniosporus	*cereus* 767
granularis	*brevis* 870
graveolens	*megatherium* 615, 872; (*subtilis* 730)
hollandicus	*brevis* 616, 874; (*cereus* 875)
immobilis	(*megatherium* 1024)
krzemieniewski	*circulans* 760
lacticola	*cereus* 617, 731, 877; (*pumilus* 878)
lactimorbus	*sphaericus* 732; (*cereus* 733)
lactis	*cereus* 618, 879, 880, 881
lactis niger	*cereus* 256, 654; *subtilis* 255; *subtilis* var. *niger* 254, 655
laterosporus	*laterosporus* 314, 340, 347, 590, 882; (*cereus* 589)
lautus	*circulans* 666 (also contained *cereus*)
lentus	*lentus* 670, 883
limnophilus	*brevis* 887
liodermis	*pumilus* 734
loehnisii	*sphaericus* var. *loehnisii* 672
luteus	*cereus* 886; *subtilis* 619; *pumilus* 885
macerans	*macerans* 277, 888
maculatus	*brevis* 889
malabarensis	*megatherium* 239; (*pumilus* 620, 891)
megatherium	*megatherium* 234, 308, 892, 893, 894, 895; (*pumilus* 735, 736; *subtilis* 737)
mesentericus	*pumilus* 236, 307, 738, 896
mesentericus flavus	*pumilus* 333, 739
mesentericus fuscus	*subtilis* 897, 898
mesentericus hydrolyticus	*subtilis* 901
mesentericus ruber	*subtilis* 900; (*cereus* 644)
mesentericus viscosus	(*cereus* 903)
metiens	*cereus* 621
minimus	*subtilis* 773
modestus	*coagulans* 905
montanus	*brevis* 622
musculi	*megatherium* 623, 907; (*subtilis* 909)
mycoides	*cereus* var. *mycoides* 233, 306, 911, 912; (*brevis* 910)
natto	*subtilis* 351, 352
niger	*subtilis* var. *niger* 220, 254, 264, 650, 651, 655; (*subtilis* 913; *megatherium–cereus* intermediate group A, 656; *pumilus* 657, 658)
nigrificans	*subtilis* var. *aterrimus* 624 (mixed with *pumilus*), 659, 740; (*pumilus* 647)

Name as received	Identification
nitidis	*circulans* 625
nitroxus	(*subtilis* 918)
orae	(*subtilis* 919)
orpheus	*laterosporus* 661, 681, 682
oxalactis	*cereus* 922
oxalaticus	*megatherium* 627, 923; (*cereus* 922)
pabuli	*circulans* 924
pallidus	*brevis* 628
panis	*subtilis* 315, 332, 926; (*pumilus* 316)
para–alvei	*alvei* 685, 686
parvus	*pumilus* 629, 928
pasteurii	*pasteurii* 673, 674, 675
petasites	*megatherium* 343, 931; (*subtilis* 338, 930, 933)
petroselini	*cereus* 934
polymyxa	*polymyxa* 279, 354
praussnitzii	*cereus* var. *mycoides* 936; *cereus* 935
pseudotetani	(*sphaericus* 719)
pseudotetanicus	(*pumilus* 937)
pumilus	*pumilus* 272, 630, 939, 940; (*subtilis* 741, 941; *cereus* 942)
rarerepertus	Unclassified 943
rarus	*brevis* 632
robur	*cereus* 946, 947; (*subtilis* 631)
rotans	*sphaericus* var. *rotans* 633
rufescens	*brevis* 948
rugulosus	*subtilis* 949
ruminatus	*megatherium* 951, 952; (*pumilus* 345; *subtilis* 634)
santiagensis	*brevis* 953
scaber	*subtilis* 774, 775
segetalis	*brevis* 635
serositidis	*sphaericus* 668
serrulatus	*subtilis* 955
sessilis	*cereus* 768
siamensis	*cereus* 201
silvaticus	*megatherium* 957, 958; (*subtilis* 636)
simplex	*megatherium* 960; *megatherium–cereus* intermediate group B, 962; group C, 335, 346, 961; (*sphaericus* 349; *subtilis* 959)
sphaericus	*sphaericus* 344, 348, 966, 967; (*megatherium–cereus* intermediate group B, 964, 965; *subtilis* 963)
sublustris	*pumilus* 980
subtilis	*subtilis* 231, 243, 304, 560, 743, 744, 745, 968, 969, 971, 972, 975, 978; (*cereus* 303, 537, 538, 970, 973, 974, 976)
subtilis niger	*subtilis* var. *niger* 704
subtilis var. *viscosus*	*subtilis* 979
supraresistans	*subtilis* 977
tardivus	*subtilis* 981
tenuis	*subtilis* 776, 777
teres	*megatherium–cereus* intermediate group B, 986; *pumilus* 637; *subtilis* 987
terminalis	*circulans* 746; (*subtilis* 983)
terminalis var. *thermophilus*	(*pumilus* 984)
terrestris	(*pumilus* 638)
thermoacidurans	*coagulans* 770
thermophilus	(*pumilus* 982)
thuringensis	*cereus* 996
tritus	*pumilus* 667
tropicus	*cereus* 793
truffauti	(*pumilus* 355, 997)
tumescens	*megatherium* 991, 994, 995; (*pumilus* 990; *subtilis* 989; *cereus* 992, 993)

Name as received	Identification
tyrosinogenes	*subtilis* var. *aterrimus* 353
undulatus	*cereus* 998, 999
validus	*circulans* 639
valinovorans	*brevis* 640
ventricosus	*brevis* 641
vulgatus	*subtilis* 237, 238, 329, 787, 1003, 1004
vulgatus hydrolyticus	*subtilis* 747
watzmannii	(*subtilis* 642)

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